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Antimicrobial Resistance of *Staphylococcus aureus*: Genetic Basis

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INTRODUCTION

In recent years, there has been a dramatic increase in the incidence of hospital-associated (nosocomial) infections caused by strains of *Staphylococcus aureus* that are resistant to multiple antibiotics; some strains now demonstrate resistance to as many as 20 antimicrobial compounds, including antiseptics and disinfectants. The threat to patient care posed by such organisms, largely due to their intransigence to antimicrobial chemotherapy, has stimulated efforts to gain insights into the genetic nature of the determinants encoding antibiotic resistance and the mechanisms by which they spread in staphylococcal populations. Attention is also being directed towards understanding the processes and pathways for the evolution of resistance in *S. aureus*.

These studies have benefited, significantly, from the application of the powerful techniques of molecular genetics and have led to an explosion in the information on staphylococcal resistance since the last review devoted to this topic appeared in this journal (255). In this article, we have placed greatest emphasis on the molecular and genetic nature of resistance determinants but have also included brief descriptions of the resistance mechanisms they encode. Recent reviews which include some discussion of the genetics of antibiotic resistance in staphylococci are those by Foster (142), Poston and Naidoo (392), Brunton (55), Lacey (259), Kayser et al. (223a), and Gillespie and Skurray (157).

Emergence of Multiresistant *S. aureus*

Staphylococci, together with pneumococci and streptococci, are members of a group of invasive gram-positive pathogens, known as the pyogenic cocci, which cause various suppurative or pus-forming diseases in humans and other animals. Staphylococci are characteristically nonmotile, catalase-positive facultative anaerobes, which grow in "grapelike" clusters and can be divided into pathogenic and relatively nonpathogenic strains on the basis of the synthesis of the enzyme coagulase (19, 360). Coagulase-positive strains, classified as *S. aureus*, often produce a yellow carotenoid pigment (which has led to them being colloquially referred to as "golden staph") and cause acute to chronic infections including pimples, boils, deep tissue abscesses, enterocolitis, bacteriuria, osteomyelitis, pneumonia, carditis, meningitis, septicemia and arthritis. Strains that do not produce coagulase, such as *S. epidermidis*, are nonpigmented and are generally less invasive but have increasingly been associated, as opportunistic pathogens, with serious nosocomial infections (8, 363). For reviews of biological, clinical, and epidemiological aspects of staphylococci, see references 117 and 118.

Before the antibiotic era of medicine began some 40 years ago, the prognosis for patients with severe staphylococcal infections was extremely poor. The introduction of penicillin into clinical use in the early 1940s, though, brought about a dramatic reversal in this situation (386). For the first time, invasive *S. aureus* infections, such as those that develop from accidental or operative trauma, burns, and other serious skin lesions, could be treated effectively.

The period of universally effective penicillin therapy was disconcertingly short-lived, however. Within a few years the appearance of penicillin-resistant strains of *S. aureus* was reported (21, 343), and by 1946 it was estimated that 60% of hospital isolates in the United Kingdom were resistant to penicillin (23). The successive introduction of streptomycin, tetracycline, chloramphenicol, and the macrolides (e.g., erythromycin) was similarly attended by the emergence of resistant organisms (386, 448). Somewhat ominously, the strains which had acquired resistance to these new antibiotics were also usually resistant to penicillin through the production of a β-lactamase (penicillinase). The result was the creation of organisms with a wide spectrum of resistance and a marked ability to survive and be spread in the hospital environment (368). Such multiply antibiotic-resistant *S. aureus* strains were of global significance during the 1950s (367, 418, 448); in one hospital in the United States >40% of the *S. aureus* isolates from in-patients during 1959 were found to be resistant to four or more antibiotics (59).

The introduction of the semisynthetic β-lactamase-resistant penicillins, such as methicillin and oxacillin, brought about a general decline in the prevalence of multiply resistant *S. aureus* during the early 1960s (448). Although resistance to methicillin was detected in approximately 1% of isolates from the United Kingdom, such strains did not pose a serious threat to the overall effectiveness of the antibiotic (369). By the late 1960s to early 1970s, however, strains resistant to the semisynthetic β-lactams were isolated with increasing frequency in a number of countries including Australia (420, 421), Belgium (237), France (90), Poland (41), the United Kingdom (369), and the United States (25, 362); in Denmark (60) and Switzerland (223, 225), these strains formed a significant proportion of the *S. aureus* isolates. Most methicillin-resistant strains isolated at this time produced a β-lactamase and were also resistant to streptomycin, sulfonamides, and tetracycline; many demonstrated additional resistance to chloramphenicol, erythromycin, and fusidic acid or to the aminoglycoside, neomycin (255).

Neomycin-resistant *S. aureus* strains had first been detected in the United States in 1959 to 1960 (138, 396) and soon appeared elsewhere (215, 409, 419). Resistance to neomycin, and to the related aminoglycosides kanamycin and paromomycin, had emerged after a delay of approximately 10 years and was attributed to the widespread topical use of neomycin on the skin and in the nose (290, 419). A similar delay occurred between the introduction of the aminoglycoside gentamicin in 1964 and outbreaks of hospital infection in 1975 to 1976 caused by *S. aureus* strains concomitantly resistant to gentamicin and two other aminoglycosides, kanamycin and tobramycin (38, 58, 310, 471, 552); once again, extensive topical use of the antibiotic was implicated in the appearance and spread of these resistant strains (341, 552). Prior to these outbreaks, gentamicin-resistant strains were rare, being obtained only as single isolates in France (470), the United Kingdom (268, 390), and the United States (385). Reports of *S. aureus* strains resistant to both gentamicin and methicillin, along with a wide range of other antibiotics including penicillin, tetracycline, and streptomycin, initially came from Melbourne, Australia (378), and London, England (450), in 1976, although similar strains were subsequently reported to have been present in Dublin, Republic of Ireland (63), and in the United States (95) at that time.

During the late 1970s and early 1980s, strains of *S. aureus* resistant to multiple antibiotics including methicillin and gentamicin were increasingly responsible for outbreaks of hospital infections in countries around the world, e.g., Argentina (440), Austria (472), Australia (152, 234, 376, 377, 508), Belgium (558), Denmark (133, 414), Republic of Ireland (63, 64, 192), England (44, 393, 449), France (125, 166), East and West Germany (339, 549), Greece (151), Italy (525), Japan (242), and the United States (94, 184, 437, 538). In many instances, these outbreaks were associated with indi-

vidual wards or units, with neonatal (152, 183, 393), intensive care (498), and burns units (17, 42) being particularly susceptible. In some instances, however, the persistence and propensity for spread of these organisms posed a much more serious clinical problem, involving numerous patients at several different hospitals, as in Dublin (64), Melbourne (376), and New York (437). The organisms involved often possessed the capacity to counter almost all of the antimicrobial agents available to the clinician for the treatment of severe staphylococcal disease.

Genetic Nature of Antimicrobial Resistance

The appearance of antibiotic-resistant staphylococci over the past 40 years has been regarded as an inevitable genetic response to the selective pressure imposed by antimicrobial therapy. The often rapid emergence of resistance following the introduction of an antibiotic into clinical use illustrates the ability of microbial populations to readily adapt to changes in their environment (407, 432). Advances in the field of bacterial genetics have shed much light on the underlying genetic factors which have resulted in the emergence of these antibiotic-resistant bacteria.

When antibiotic resistance was first encountered among bacteria, including *S. aureus*, it was believed to arise solely by mutation and selection. Spontaneous bacterial mutants resistant to certain antibiotics can be generated at frequencies of 10^{-6} to 10^{-8} per cell in the laboratory, and it was assumed that analogous events had occurred in natural populations to produce resistant organisms (432). Indeed, resistance within the staphylococci to several therapeutically useful antibiotics, including streptomycin, rifampin, fusidic acid, and novobiocin, is thought to be derived by chromosomal mutation (259). A number of resistance determinants have been mapped on the *S. aureus* chromosome (Fig. 1).

In some cases, however, chromosomal point mutations which lead to antibiotic resistance can be deleterious to the organism, resulting in the creation of less virulent forms (432). The acquisition of new characters, without affecting the fitness of the bacteria to survive in their natural environment, would therefore be expected to occur over a substantial time span. In an evolutionary sense, then, the accumulation of chromosomal mutations would seem to be unsatisfactory as the sole explanation for the rapid emergence of multiresistant bacteria (29).

The relatively minor role played by spontaneous mutation in the sudden appearance of antibiotic-resistant microorganisms was confirmed by the discovery of gene transfer and the demonstration that bacteria can acquire additional genetic material in the form of extrachromosomal or plasmid deoxyribonucleic acid (DNA) (for reviews, see references 46 and 134). The existence of plasmid DNA molecules was suggested by the transfer of discrete genetic units of resistance between bacterial strains and the irreversible loss of such units from cells at relatively high frequencies. During the last two decades, evidence has accumulated indicating that, in many instances, resistance to antimicrobial agents in the staphylococci, as in the gram-negative bacteria, is due to the presence of plasmids that carry the genetic determinants of resistance (for reviews, see references 157, 255, 348, and 392). These plasmids have been organized into 13 incompatibility groups on the basis of their inability to coexist in the same cell (211, 392).

Several factors are believed to contribute to the success of plasmid-encoded antibiotic resistance as an evolutionary

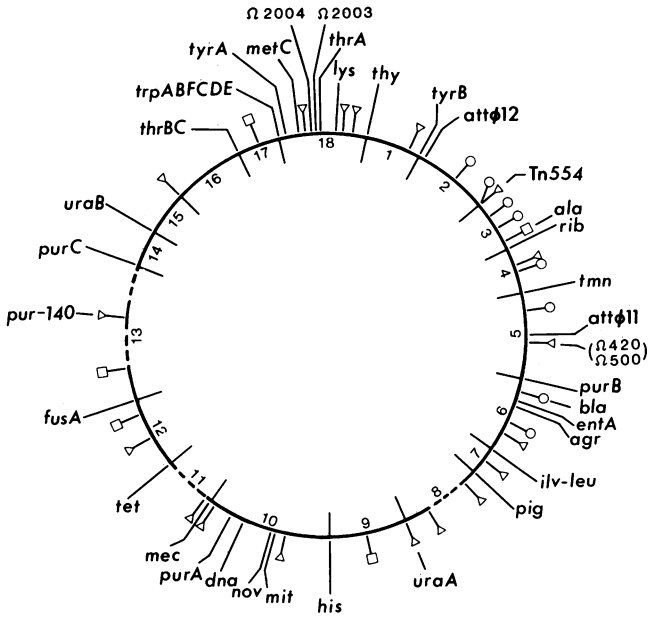

FIG. 1. Chromosome map of *S. aureus* as determined by Pattee and co-workers (373; Pattee et al., submitted). Genetic markers are represented by: *agr*, extracellular toxins regulator; *ala*, L-alanine requirement; *attϕ11*, prophage ϕ11 integration site; *attϕ12*, prophage ϕ12 integration site; *bla*, β-lactamase production; *dna*, temperature-sensitive DNA synthesis; *entA*, enterotoxin A production; *fusA*, fusidic acid resistance; *his*, L-histidine requirement; *ilv-leu*, L-isoleucine, L-valine, L-leucine requirement; *lys*, L-lysine requirement; *mec*, resistance to methicillin; *metC*, methionine requirement; *mit*, mitomycin C, nitrosoguanidine, and ultraviolet sensitivity; *nov*, resistance to novobiocin; *pig*, absence of golden-yellow pigment; *purA*, *purB*, *purC*, and *pur-140*, purine requirements; *rib*, riboflavin requirement; *tet*, resistance to tetracycline; *thy*, thymine requirement; *thrA*, *thrB*, and *thrC*, L-threonine requirements; *tmn*, resistance to tetracycline and minocycline; Tn*554*, insertion site for Tn*554*; *trp*, L-tryptophan requirement; *tyrA* and *tyrB*, L-tyrosine requirements; *uraA* and *uraB*, uracil requirements; Ω420 and Ω500, insertion sites of pI258 plasmid; Ω2003 and Ω2004, insertion sites of Tn*551* that impair *mec*-conferred resistance to methicillin. Sites of representative Tn*551* (▽), Tn*916* (□), and Tn*4001* (○) (Mahairas et al., submitted) chromosomal insertions are also shown.

mechanism (432). The presence of antibiotic resistance genes on plasmids, which are normally nonessential for the survival of the organism, provides the bacterial population with a means to reduce the genetic and physiological load on the majority of cells while, through the carriage of plasmids, a minority of cells are able to maintain the genetic diversity of the population (78). Plasmid-borne genes can undergo more radical evolutionary changes without affecting the viability of the cell, as would changes to indispensable chromosomal genes, and established plasmid transfer mechanisms can provide recipient cells with new genetic material which has already been refined by selective pressures elsewhere (29).

Plasmids can, however, contribute to the development of chromosomal resistance in two ways. First, plasmids, either in part or in toto, can integrate into the bacterial chromosome. In the case of plasmids from gram-negative bacteria, this may involve short segments of DNA, termed insertion sequences (IS), which reside on both plasmid and chromosome and provide limited regions of DNA sequence homology for recombination (see references 46, 86, and 201).

TABLE 1. *S. aureus* antimicrobial resistance genes[a]

Gene	Resistance to[b]:	Mechanism of resistance[c]	Location[d]
aacA-aphD	Gm Tm Km	AAC(6′)APH(2″)	P, P::Tn, C::Tn
aadA	Sm Sp	AAD(3″)(9)	P
aadE	Sm	AAD(6′)	P
aadD	Nm Km Pm Tm Ak	AAD(4′)(4″)	P
aphA	Nm Km	APH(3′)III	P, C, (Tn?)
aphC	Sm	APH(3″)	P
asa	Asa	Efflux	P, C::P, (Tn?)
asi-ant	Asi Sb		P, C::P, (Tn?)
bis[e]	Bi		P
bla	Pc	β-Lactamase	C, (Tn?)
blaZ (penP)	Pc	β-Lactamase	P, C::P, P::Tn, C::Tn
cadA	Cd Zn	Efflux	P, C::P
cadB	Cd Zn (low level)	Ion binding (?)	P
cadC	Cd (low level)	Efflux	C, (P?)
cat	Cm	CAT	P
dfrA	Tp	DHFR with reduced affinity	P, (Tn?)
dfrB[e]	Tp (low level)	Overproduction of DHFR (?)	C
ermA	MLS (Em)	rRNA methylation	C::Tn
ermB	MLS (Em)	rRNA methylation	P::Tn
ermC	MLS (Em)	rRNA methylation	P
fusA	Fa	G factor with reduced affinity (?)	C
fusB[e]	Fa	Decreased permeability (?)	P
lea[e]	Pb		P, C::P
mec	Mc	PBP with reduced affinity	C::Tn
merA	Hg	Mercuric reductase	P, C::P, (Tn?)
merB	Om	Organomercurial lyase	P, C::P, (Tn?)
nov	Nv	DNA gyrase with reduced affinity (?)	C
qacA	Ac Eb Qa Pi Dd	Efflux	P
qacB	Ac Eb Qa	Efflux (?)	P
qacC	Eb Qa	Efflux	P
rif	Rf	RNA polymerase with reduced affinity (?)	C
sga	Sg_A	Streptogramin *o*-acetyltransferase	P, C
sgb	Sg_B	Streptogramin hydrolase	P
spc	Sp	AAD(9)	C::Tn
strA	Sm	Ribosomal alteration	C
strB[e]	Sm (low level)		C
sulA	Su	Overproduction of *p*-aminobenzoic acid	C
sulB[e]	Su	DHPS with reduced affinity (?)	P
tet	Tc	Efflux (?)	P, C::P
tmn	Tc Mn	Efflux (?)	C

[a] See relevant sections of the text for discussion and references.

[b] Abbreviations for antimicrobial agents: Ac, acriflavine; Ak, amikacin; Asa, arsenate; Asi, arsenite; Bi, bismuth; Cd, cadmium; Cm, chloramphenicol; Dd, diamidinodiphenylamine dihydrochloride; Eb, ethidium bromide; Em, erythromycin; Fa, fusidic acid; Gm, gentamicin; Hg, mercury; Km, kanamycin; Mc, methicillin; MLS, macrolides, lincosamides, and streptogramin B; Mn, minocycline; Nm, neomycin; Nv, novobiocin; Om, organomercurials; Pb, lead; Pc, penicillin; Pi, propamidine isethionate; Pm, paromomycin; Qa, quaternary ammoniums; Rf, rifampin; Sb, antimony; Sg_A, streptogramin A; Sg_B, streptogramin B; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamides; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim; Zn, zinc.

[c] In those instances marked (?) the mechanism of resistance is uncertain.

[d] Abbreviations for gene locations: C, chromosome; P, plasmid; Tn, transposon; C::P, plasmid integrated into chromosome; C::Tn, transposon integrated into chromosome; P::Tn, transposon integrated into plasmid.

[e] The existence of this gene is suggested but remains to be confirmed.

Second, plasmids, together with bacteriophages, can act as vectors for transposable DNA elements or transposons (238). Antimicrobial resistance genes carried by transposons can be translocated either from one plasmid to another or from a plasmid to a chromosomal site, irrespective of extensive genetic homology. Moreover, events involving transposons can occur independently of the host's general recombination (*rec*) system by a mechanism(s) termed site-specific recombination. Such genes may therefore become established in diverse species in which the vector molecules themselves cannot replicate (282). In addition to facilitating the spread of antimicrobial resistance, the clustering of transposons carrying different resistance determinants on plasmids, or the chromosome, provides an explanation for the emergence of multiresistant bacteria (86, 432).

A summary of the known resistance determinants in *S. aureus*, the phenotypes they encode, the mechanisms whereby they exert resistance, and the nature of their encodement is provided in Table 1.

TRANSFER OF RESISTANCE GENES IN STAPHYLOCOCCI

The genetic exchange of resistance determinants between organisms of the same or different species is believed to play a crucial part in the evolution of antibiotic-resistant bacteria (for reviews, see references 134, 407, and 432). As in other genera, several mechanisms exist for the transfer of genetic material between staphylococci. These are the traditional processes of transduction, transformation, and conjugation and a process referred to as mixed-culture transfer or "phage-mediated conjugation." Although these mechanisms have been demonstrated in the laboratory, the contribution each makes to the exchange of resistance determinants in the natural environment is unknown.

As with other bacteria, the ability of a staphylococcal strain to act as a recipient for transfer of resistance can be influenced by the presence of restriction systems encoding enzymes which degrade incoming, unmodified DNA (319). Restrictionless mutants of some *S. aureus* strains exist (210, 483) and have been used as recipients in laboratory studies (131, 141, 296).

Staphylococcal Bacteriophages and Transduction

Staphylococcal bacteriophages have long played an important role, not only in transductional genetic analysis (see references 255 and 392 for reviews), but also in the subdivision and classification of *S. aureus* strains for epidemiological purposes. The latter is achieved on the basis of the response of a strain to the International Basic Set of 23 typing phages (367). The four major phage groups of human *S. aureus* strains are groups I, II, III, and a miscellaneous group, the members of which respond to one or more distinctive phages; more complex patterns, such as reaction to both lytic group I and III phages, are commonly reported (367). This grouping of strains is considered to result, at least in part, from differences in restriction-modification systems (483, 524); for example, all phage group II strains tested, but no strain from phage group I or III, produced an enzyme with *Sau*3AI endonuclease activity (482). Many recent isolates of multiresistant *S. aureus* were nontypable by a routine test dilution of phage of the International Basic Set, often due to the presence of restriction nucleases, and although some strains demonstrated a weak response to phage at concentrations 10× or 100× the routine test dilution, others could only by lysed by "experimental" phage not formally included in the International Basic Set (12, 27, 63, 64, 264, 293, 438, 526). A historical account tracing the emergence of strains of specific phage groups, and their antibiotic characteristics, has been provided by Parker (367).

The current Basic Set of human *S. aureus* typing phages contains representatives of three serological groups, A, B, and F (413). There are few reports of molecular comparisons of phage within the same lytic or serological group (365, 478).

The majority of clinical isolates possess one or more temperate phages, presumably as prophages integrated into the chromosome, the presence of which may also affect the phage-typing pattern (27, 417, 544); lysogenicity patterns combined with phage types have been used for epidemiological and evolutionary comparisons of clinical isolates (27, 526a). The lysogenic status of a strain has also been reported to affect its ability to engage in gene transfer via transformation (422), transduction (81, 82, 84; however, see reference 476), and mixed culture (251, 256).

For many years, transduction was believed to be the only mechanism by which staphylococci could exchange genetic information in vivo (255, 256). For transduction to occur, transducing phage particles need to be produced from the donor strain through infection with a lytic bacteriophage or by the induction of a prophage. Calcium ions are required for the attachment of phage particles to cell surfaces, and so transduction, as well as lytic phage activity, can be inhibited by the presence of chelating agents such as citrate ions. In the main, staphylococcal transducing phages, which include the typing phages 29, 80, 53, and 88 together with φ11, belong to the serological group B phage (255), although transduction of gentamicin resistance has been associated with a group F phage (104).

Where examined, staphylococcal transducing phage have genome sizes of approximately 45 kilobase pairs (kb) (18, 289, 366, 478). This limitation on the amount of DNA that can be packaged in the phage head could explain the much higher transduction frequencies obtained with plasmids of 30 to 40 kb or less (72, 308, 423) and may be the rationale for the majority of staphylococcal plasmids having molecular sizes of <45 kb. Comparisons of lytic phage particles and transducing phage particles bearing chromosomal (34) or plasmid (518) determinants revealed little if any difference in density, indicating that infectious and transducing particles contain similar quantities of DNA. Particles transducing small plasmids must therefore contain DNA in excess of one plasmid genome, and it has been demonstrated for the 2.9-kb chloramphenicol resistance (Cm^r) plasmid pC194 (111) and the 4.4-kb tetracycline resistance (Tc^r) plasmid pT181 (349a) that these plasmids resided in the phage particles as linear concatamers. These multimeric forms are generated via a mechanism that depends on plasmid-initiated replication, rather than plasmidic recombination, and are resolved into monomers after transfer, again by replicative processes (349a).

The cotransduction of small staphylococcal plasmids has been shown with streptomycin resistance (Sm^r) and Tc^r plasmids (176, 177) and subsequently with a variety of other plasmids (209, 243, 480). It was suggested that these plasmid pairs underwent a recombination-dependent linkage during formation of the transducing particle which was readily reversible in the recipient cell, although cotransduced plasmids occasionally failed to resolve on transfer and remained associated as stable plasmid cointegrates (212, 351, 357). Evidence has been presented that some cointegrates are formed through the action of host- and phage-mediated recombination systems on short regions of homology, designated recombination sites (RS), which occur on each of the participating plasmids (351, 352, 357).

Whereas the generalized transducing phages of *S. aureus* are able, as their name implies, to transfer chromosomal or plasmid DNA, a derivative of φ11 is not. This phage, φ11de, which arose by in vivo recombination between φ11 and the penicillin and erythromycin resistance (Pc^r, Em^r) plasmid pI258, acts more as a specialized transducing phage in being able to transfer the integrated Em^r determinant at high frequencies; φ11de replicates as a plasmid through the presence of the pI258 replicon and is defective as a virus (345). More recently, it has been shown that φ11de is able to transduce the Cm^r plasmid pC194, but not the neomycin-kanamycin resistance (Nm^r Km^r) plasmid pUB110 (112), and that particles able to cotransduce Cm^r (pC194) and Em^r (φ11de) contained DNA composed of a linear multimer of approximately five copies of pC194 inserted randomly into the φ11 genome (111).

Transformation

The transformation of *S. aureus* with "naked" plasmid or chromosomal DNA can be achieved in the presence of high concentrations of calcium ions with recipient cells that have attained competence (285, 286, 422). A narrow peak of competence occurs prior to exponential growth due to the absence of extracellular deoxyribonuclease; the presence of deoxyribonuclease is believed to preclude the transfer of DNA by transformation under natural conditions (422). Competence is also dependent on the existence of competence-conferring factors provided by superinfecting bacteriophage or induced prophage. In the case of the widely used recipient strain NCTC 8325, the natural prophage φ11 possesses competence enhancement ability as either a

prophage (422) or added lysate (462); superinfection with serological group B typing phages is also effective (496). The uptake of DNA is facilitated by the adsorption to cells of the competence-conferring factor made up of proteinaceous subvirion components (497), which, when viewed by electron microscopy, gave the appearance of aggregated phage tails (39, 214).

Transformation has been used for the introduction of *S. aureus* plasmids or their derivatives into staphylococci (287, 288, 542, 543), *Bacillus subtilis* (68, 119, 120, 137, 178, 287), and *Escherichia coli* (167) for possible use as molecular cloning vectors. *S. aureus* has also been transformed by plasmid DNA isolated from *B. subtilis* and *S. epidermidis*, the restriction barrier being overcome by the use of a restriction-negative mutant or by heating the recipient culture to inactivate nucleases (461).

The construction of genetic linkage maps of the *S. aureus* chromosome has also made use of transformational analysis (374). More recently, the transfer of *S. aureus* plasmid DNA by protoplast transformation (334) and protoplast fusion (164) has been demonstrated, and use of these techniques for the transfer of chromosomal markers has, for the first time, enabled the establishment of a circular map of the *S. aureus* chromosome (Fig. 1) (474, 475).

Mixed-Culture Transfer and Phage-Mediated Conjugation

The relatively low frequency of transfer by transduction, combined with the requirement for death of the donor cell, and the need to protect recipient cells against lytic phage particles by lysogeny prompted the suggestion that transduction has a limited role in the transfer of genetic material in vivo (255, 405), although it is clear that some transfer in mixed cultures of donor and recipient cells does occur by this mechanism (256, 318, 353, 549). The observations that resistance determinants encoded on plasmids can be transferred in mixed cultures at very high frequencies ($>10^{-1}$ resistant transcipients per final donor cell) following overnight incubation (256) and that chromosomal resistance genes can also be transferred, although at somewhat reduced frequency (254), have led to the proposal of an alternative mechanism to account for such efficient gene transfer in mixed cultures of donor and recipient cells (255, 256). This process has been referred to as phage-mediated conjugation by Lacey (256) because there is a requirement for the presence of a prophage in either the donor or the recipient, together with high cell density and calcium ions (251, 252, 545, 546).

The mechanism of phage-mediated conjugation is unlike transduction in that the donor need not be lysogenic, and transfer cannot be achieved from a phage lysate of the donor (256, 435). Little spontaneous phage activity is found in the broth cultures, and filtrates do not necessarily contain transducing particles. The nontransductional nature of this mechanism is further supported by the finding that transfer proceeded between donor and recipient cells when either was lysogenized with a φ11 mutant that was unable to form phage heads (547). Although the exact nature of prophage involvement is not known, Lacey (256) postulated that the bacteriophage could be instrumental in altering the adhesiveness of the cell surface, thereby enhancing the clumping of cells and enabling plasmid DNA to pass directly from donor to recipient. The stimulation of transfer by a mutant phage, either as a lysogen or when added as a lysate to the mixed culture, suggests that only some component(s) of the phage is required to facilitate cell-cell contact (547); this may prove to be the same phage component which confers competence for transformation on the recipient. Recently, Barr et al. demonstrated that the phage-mediated conjugational transfer of a Tc^r plasmid was enhanced 100- to 1,000-fold in the presence of subinhibitory concentrations of β-lactam antibiotics and suggested that such antibiotics may increase aggregate formation between donor and recipient cells (24a).

Further evidence for a conjugative process in staphylococci includes the ability to transfer antibiotic resistance to encapsulated strains which are unable to engage in transduction (545), the failure of deoxyribonuclease (435, 545), gamma globulin (54, 546), antiphage serum (143), or pronase (435) to inhibit transfer, and the simultaneous transfer of up to three different antibiotic resistance plasmids at high frequency (143, 256, 546).

Plasmid-Mediated Conjugational Transfer

Gentamicin resistance (Gm^r) plasmids which encode their own transfer by a conjugationlike mechanism have been reported in staphylococcal isolates from widely separated geographical locations (11, 141, 159, 308, 339, 434, 509; W. Witte, personal communication). Such self-transmissible (Tra^+) plasmids ranged from 38 to 57 kb in size and could be transferred between apparently nonlysogenic strains of *S. aureus* either at relatively low frequencies in broth cultures or more efficiently by filter mating on agar medium (11, 308). Filtrates of donors and transconjugants exhibited no phage activity, and the addition of deoxyribonuclease, citrate, ethylenediaminetetraacetic acid, calcium chloride, or human sera to the mating mixes did not affect transfer (11, 141, 308). In view of these characteristics, and the requirement for viable cell-to-cell contact, the mechanism of transfer was concluded to be a conjugationlike process. In support of the idea that cell-cell communication is a prerequisite for transfer of the plasmids, the addition of polyethylene glycol to broth cultures has been shown to increase cell aggregation with a proportional increase in transfer of the Tra^+ plasmid (509). Tra^+ plasmids are also able to mobilize or cotransfer smaller coresident antibiotic resistance plasmids which are not independently transferable (11, 141, 158, 308, 509), thereby suggesting a further significant role for plasmid-mediated conjugation in the dissemination of resistance among staphylococcal populations.

All transmissible staphylococcal plasmids isolated to date encode gentamicin resistance and many also encode resistance to other aminoglycosides, ethidium bromide, and quaternary ammonium compounds, while some also encode resistance to penicillin and trimethoprim. Comparative analysis of a number of these plasmids, isolated from both *S. aureus* and *S. epidermidis*, has demonstrated that many are closely related (10, 160, 308, 434) and presumably share a common region involved in transfer. Restriction endonuclease analysis of Tra^- deleted plasmids has localized the genes for conjugative transfer within a 12- to 15-kb region of plasmid DNA (see Fig. 9) (13, 308). While the precise nature of the conjugal process in staphylococci has yet to be resolved, it cannot be as complex as the "classical" conjugative transfer mechanism operative in gram-negative organisms, which requires pilus contact and some 33 kb of DNA in the case of the F plasmid (540). More likely, conjugative mechanisms in the staphylococci will be shown to resemble those active in the streptococci (75, 77, 246).

Both phage-mediated and plasmid-mediated conjugation provide attractive mechanisms for the transfer of antibiotic resistance determinants among staphylococci under natural

conditions. The transfer of resistance determinants in mixed cultures on the skin of human volunteers has been demonstrated (216, 252, 269, 335, 337, 338), thereby suggesting the importance of the body surface, the normal staphylococcal habitat, in the emergence of antibiotic-resistant organisms (341). Furthermore, plasmid-mediated conjugation has been reported to be stimulated in the presence of dry absorbent surfaces; therefore, transfer of resistance might also be expected to take place on surgical dressings, clothing, and bedding in the hospital environment (509a, 510).

RESISTANCE TO β-LACTAM ANTIBIOTICS

The β-lactam antibiotics or penicillins produce a bactericidal effect by inhibiting the membrane-bound enzymes responsible for catalyzing vital stages in the biosynthesis of the cell wall. Such inhibition is the direct result of the covalent binding of the antibiotic to one or more penicillin-sensitive enzymes, termed penicillin-binding proteins (PBPs); for recent reviews, see references 145, 399, 400, 473, and 530.

There are three clinically significant mechanisms of resistance to the β-lactam antibiotics in *S. aureus*. The first, covered in this section, is the inactivation of the penicillin through β-lactamase- or penicillinase-mediated hydrolysis of the β-lactam ring of the antibiotic. The second mechanism, which is equally effective against β-lactamase-sensitive and β-lactamase-resistant penicillins, including methicillin, is an intrinsic resistance involving a lowering of the affinity or the amount of the PBPs. This mechanism of penicillin resistance will be dealt with separately. The third mechanism is tolerance to the bactericidal effect of β-lactam antibiotics (for reviews, see references 425, 426, and 515a). The lysis that follows inhibition of cell wall synthesis by an antibiotic is brought about by intracellular or cell wall-associated autolytic enzymes (370). Tolerant microorganisms have reduced autolytic activity (35), and in the case of staphylococci, experiments indicate that this is due to an excess of an autolysin inhibitor (429). Hence, tolerance is manifested as an increased resistance to the lethal, rather than the inhibitory, action of β-lactams (35, 161, 306, 429); cross-tolerance to the killing effects of other cell wall synthesis inhibitors, such as vancomycin, has been reported (429). The genetic basis of tolerance has not been described, although bacteriophage have been implicated in the conversion of nontolerant organisms to tolerance (43).

β-Lactamase-Mediated Resistance to β-Lactam Antibiotics

The correlation between penicillin-resistant clinical isolates of *S. aureus* and the production of β-lactamase was first reported in 1944 (235). The staphylococcal β-lactamases are extracellular enzymes which have been divided into four types (A to D) on the basis of serological and kinetic tests (403, 412). The enzymes of serotypes A and C are of high activity and were found to be produced by typical hospital *S. aureus* strains (phage groups I and III) isolated during the 1960s, whereas the lower-efficiency B-type enzyme was produced exclusively by phage group II organisms, which had limited incidence in the hospital setting (116, 403). Although these enzymes hydrolyze benzyl penicillin (penicillin G) and ampicillin with high activity, they demonstrate poor activity against the semisynthetic penicillins such as methicillin and oxacillin. The four β-lactamases are believed to be closely related, with the small differences in hydrolysis rates being attributed to minor differences in amino acid residues (403, 412). Amino acid sequencing of the type A staphylococcal β-lactamase has demonstrated that this protein consists of a single polypeptide chain of 257 residues with an estimated molecular weight of 28,800 (3). When synthesized in cell-free transcription and translation systems, this β-lactamase had a molecular weight of approximately 32,000 (312); however, nucleotide and amino acid sequence analyses have demonstrated that the enzyme possesses a signal or leader peptide of 24 amino acids which is presumably cleaved as the protein is secreted (313).

Unlike the β-lactamases of gram-negative bacteria which are generally expressed constitutively, the staphylococcal β-lactamases, with the exception of the type D enzyme (412), are only expressed at high levels following induction by penicillin or its analogs (114, 316). Richmond (404) showed with the aid of heterodiploids that the inducible wild-type state was dominant to the mutant constitutive state and consequently proposed that β-lactamase synthesis in *S. aureus* was under the negative control of a diffusible repressor molecule. This molecule, the product of the *blaI* (*penI*) gene, is believed to be a tetramer of four similar subunits (205, 206) which binds to the operator site of the β-lactamase structural gene (*blaZ* or *penP*) and thereby inhibits its transcription (204). Nucleotide sequencing has determined that the operator site may consist of a 22-base pair (bp) inverted repeat sequence which is located within the transcriptional initiation region of *blaZ* (313).

Evidence has been presented which suggests that penicillin and its analogs function in the induction of β-lactamase synthesis by specifying the conformation of a β-lactamase antirepressor protein which subsequently inactivates the β-lactamase repressor (203). This proposal accounts for arguments against the active involvement of penicillin analogs or cell wall precursors in the inactivation of the β-lactamase repressor (204) and is supported by genetic evidence for the existence of a locus unlinked to *blaI* and *blaZ* which is involved in the regulation of β-lactamase synthesis (80, 85). The putative antirepressor protein, the product of the chromosomal R2 or *penA* gene, is constitutively synthesized in the inactive form; however, the presence of the inducer is believed to direct either the formation of or accessibility to the active site of the antirepressor molecule (204).

β-Lactamase Plasmids

The instability of penicillin resistance was noted soon after its emergence (22), but the possibility that the genetic determinant for β-lactamase production was carried by a plasmid was not recognized until the transduction experiments of Novick (344) implicated extrachromosomal inheritance. The physical isolation of circular duplex DNA molecules specifying the production of β-lactamase was reported some years later (423), and a variety of β-lactamase plasmids have subsequently been found in naturally occurring strains of penicillin-resistant *S. aureus* (2, 108, 116, 154, 169, 219, 382, 446). β-Lactamase plasmids invariably exhibit linked resistance to one or more other antimicrobial agents which may include inorganic ions such as antimony, arsenate, arsenite, bismuth, cadmium, lead, mercury, and zinc (358); organomercurial compounds such as phenylmercuric acetate (536); antiseptics, disinfectants, and dyes such as acriflavine (132, 155); ethidium bromide (155, 220) and quaternary ammonium compounds (128, 155); as well as antibiotics such as erythromycin (171, 322), fusidic acid (262), and the aminoglycosides kanamycin and gentamicin (157, 171, 512).

TABLE 2. Selected *S. aureus* β-lactamase and heavy-metal resistance plasmids and their characteristics

Plasmid	Family	Resistance to[a]:	Size (kb)	Inc group	Reference(s)
pI524	Alpha	Pc Asa Asi Sb Hg Om Cd Bi Pb	31.8	1	332, 382, 446
pI55c1	Alpha	Pc Hg Om Cd Bi Pb	29.8	1	382, 446
pI13371	Alpha	Pc Asa Asi Sb Hg Om Cd Bi Pb	31.1	1	382, 446
pUB108	Alpha	Asa Asi Sb Hg Om Cd Bi Pb	46.0	1	261, 446
pSJ1	Alpha	Pc Asa Hg Cd Em Km	40.0	1	169, 171
pSJ24	Alpha	Pc Asa Hg Cd Em Gm Km	35.8	1	169, 171
pSK23	Alpha/beta	Hg Cd Ac Eb Qa Gm Tm Km	38.7	1	157
pI836	Alpha/gamma	Pc Asa Asi Sb Hg Om Cd Bi Pb	29.1	1	106, 446
pSK57	Alpha/gamma	Pc Hg Cd Ac Eb Qa Pi Dd	28.8	1	155, 157
pWG50	Alpha/gamma	Pc Hg Om Cd Gm Tm Km Eb Qa	42.8		128, 504, 512
pI258	Gamma	Pc Asa Asi Sb Hg Om Cd Bi Pb Em	28.2	1	333, 355, 382, 446
pI6187	Gamma	Pc Asa Asi Sb Hg Om Cd Bi Pb	23.1	1	382, 446
pI9789	Gamma	Asa Asi Sb Hg Om Cd Bi Pb	19.7	1	15, 446
pRN4115	Gamma	Pc Asa Asi Sb Hg Om Cd Bi Pb	25.8	1	15, 446
pII147	Beta	Pc Asa Asi Hg Om Cd Pb	32.6	2	355, 382, 446
pII3804	Beta	Pc Hg Om Cd Pb	31.6	2	382, 446
pII6907	Delta	Asa Asi Sb Cd Bi Pb	25.3	2	382, 446
pI1071	Orphan	Pc Cd Pb	26.5	1	382, 446
pSK67	Orphan	Pc Cd	26.5	1	154
pSK77	Orphan	Pc Cd	27.2	1	154
pII1113	Orphan	Pc Cd Pb	24.1	2	446
pII1106	Orphan	Pc Cd Pb	21.3	2	446
pUB101	Orphan	Pc Hg Cd Fa	21.2	7	270, 446
pZA10		Pc Asa Asi Hg Cd Pb(EntB, $EntC_1$)[b]	56.2		2
pIP983		Cd	3.2		124

[a] See Table 1, footnote *b*, for abbreviations of antimicrobial agents.
[b] EntB, $EntC_1$: production of enterotoxins B and C_1, respectively.

Selected β-lactamase plasmids and their characteristics are listed in Table 2.

In an extensive study of 37 different plasmids isolated from various parts of the world, Shalita and colleagues (446) demonstrated the existence of four distinct families of β-lactamase plasmids, which they designated alpha, beta, gamma, and delta, together with several unrelated or "orphan" plasmids. Classification was based on molecular interrelationships as detected by *Eco*RI and *Bgl*II restriction digestion, supplemented by digestions with other enzymes and heteroduplex analysis. A detailed analysis of β-lactamase and heavy-metal resistance plasmids from strains isolated at a single Melbourne hospital between 1946 and 1981 (154) demonstrated the presence of alpha, gamma, and orphan plasmids, together with alpha/beta and alpha/gamma recombinant plasmids (Table 2; M. Gillespie and R. Skurray, unpublished data). Although naturally occurring recombinants between plasmids of the alpha and gamma families have been described previously (446), there are no previous reports of alpha/beta recombinants, which were detected in the Melbourne isolates from as early as 1947.

The prototype of the alpha family is pI524, a 31.8-kb plasmid belonging to the IncI incompatibility group, which encodes resistance to inorganic ions and organomercurials in addition to β-lactamase production (Table 2). A physical-genetic map of pI524 has been derived (332) and is presented in modified form in Fig. 2. This plasmid was found to possess a 2.2-kb region of DNA flanked by 650-bp inverted repeat sequences (IR_L and IR_R), located adjacent to the *blaIZ* loci, which undergoes a reversible *rec*-independent inversion (332). The orientation of the invertible region (inv) is determined from the position of asymmetrically located recognition sites for a number of endonucleases including *Bgl*II, *Hpa*I, *Sal*I, *Pvu*II, and *Xba*I (see Fig. 3). The appearance of four restriction fragments in less than molar quantities, following digestion with any one of these enzymes, was indicative of populations of pI524 molecules bearing inv in both the positive and negative orientations.

A second naturally occurring alpha family plasmid, pI55cl (Table 2), possessed a deletion of approximately 500 bp within the right-hand inverted repeat sequence (IR_R) of inv which did not prevent inversion; however, plasmids have been isolated which lack the entire right-hand inverted repeat (e.g., pI13371), and the inv regions of these are incapable of inversion (446). The invertible regions of all such deleted plasmids were shown to be "locked" in the one orientation, which is deemed the positive orientation (see Fig. 3). Murphy and Novick (332) postulated that, although not lacking the specific recombination enzyme system required for inversion, the locked derivatives of pI524 had apparently deleted the site of recombination, which they presumed to lie within or at the ends of the inverted repeats.

Plasmids from the alpha family were also shown to possess a characteristic region of DNA consisting of three copies of a directly repeated sequence of about 1.4 kb, separated by spacer segments of 3.1 and 1.4 kb, located between the maintenance/compatibility/replication (mcr) region and the mercury resistance (Hg^r) operon (Fig. 2) (446). The origin and possible function of this region were not determined.

Members of the gamma family of β-lactamase plasmids also belong to the IncI incompatibility group and share substantial homology with the alpha family plasmids (446). The prototype of the gamma family is pI258 (Table 2, Fig. 2), a 28.2-kb plasmid which carries the Em^r transposon Tn*551* (350). Analysis of heteroduplexes between pI258 and pI524 revealed that the two plasmids share about 23 kb of sequence homology (333). Gamma family plasmids differ from the alpha plasmids primarily by a deletion of the invertible region and the associated left-hand inverted repeat sequence and an inversion of the *blaIZ* loci and the right-hand inverted repeat sequence of inv (Fig. 2) (333). These plasmids also

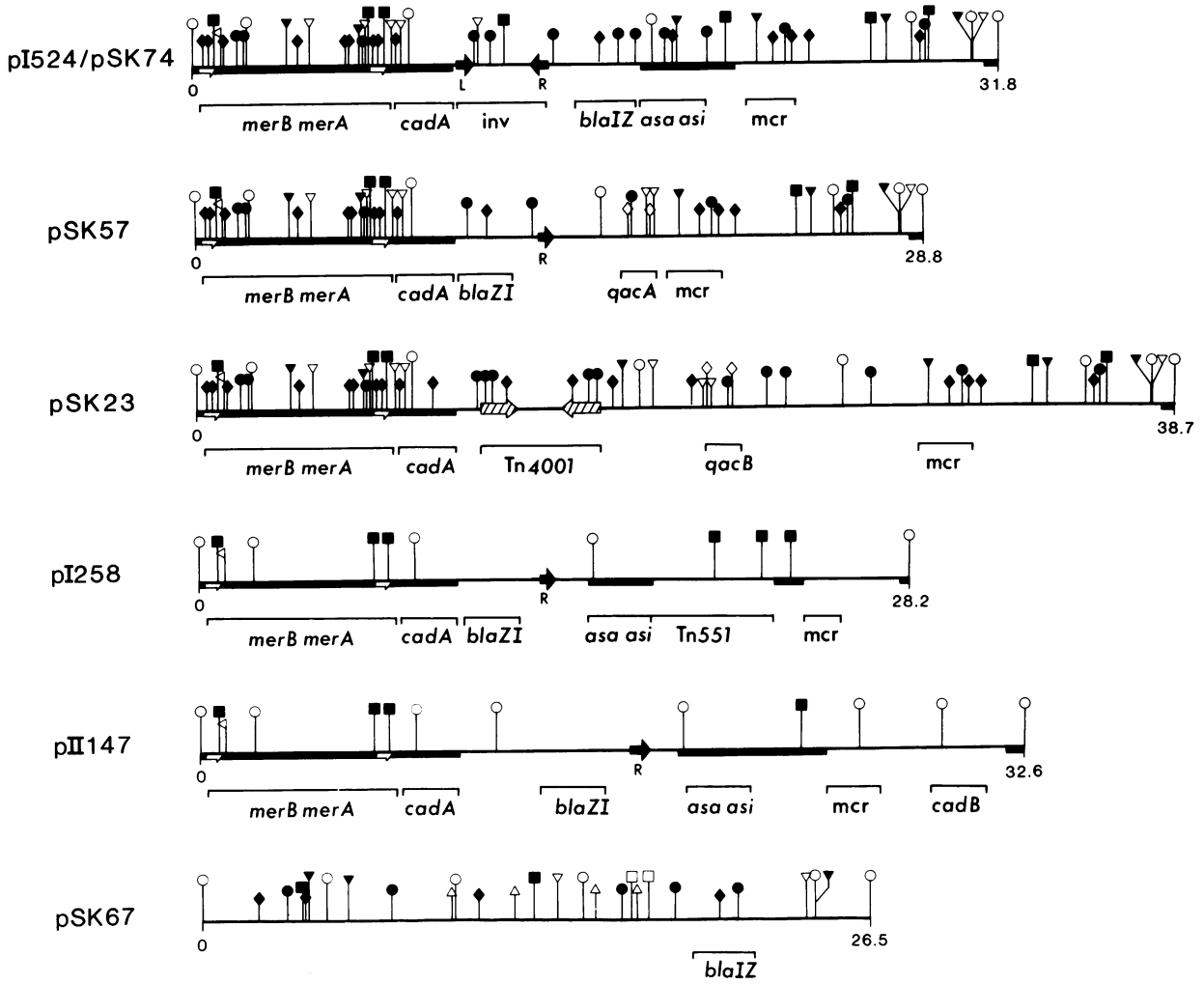

FIG. 2. Genetic and restriction maps of the β-lactamase and heavy-metal resistance plasmids pI524/pSK74, pSK57, pSK23, pI258, pII147, and pSK67 (155, 355, 446; Gillespie and Skurray, unpublished data). Regions that are conserved among these plasmids are defined by the bold lines. Restriction endonuclease sites are indicated by: ◁ (*Bam*HI), ■ (*Bgl*II), ○ (*Eco*RI), ◆ (*Hin*dIII), ● (*Hae*III), ▼ (*Pst*I), and ▽ (*Pvu*II). Coordinates are in kilobases. See Table 1 and text for definitions of genetic loci and other regions; *blaZI* indicates an inversion of the *blaIZ* region, and *asa asi* indicates the location of the *asa–asi-ant* region. Tn551, on pI258, encodes the *ermB* MLS^r determinant. Closed arrows represent the inverted repeat sequences IR_L(L) and IR_R(R) associated with the β-lactamase invertible region (inv), open arrows represent direct repeats (homologous with IS*257*) associated with the *mer* operon, and hatched arrows represent inverted repeats of IS*256* associated with the gentamicin resistance transposon Tn*4001* (see relevant sections).

lack part of the characteristic region of the alpha plasmids, containing two of the 1.4-kb directly repeated sequences (446).

Plasmids of the beta and delta families belong to the Inc2 group and are therefore compatible with the alpha and gamma plasmids. The prototype of the beta family, pII147 (Table 2, Fig. 2), shares approximately 72% base sequence homology with pI258, despite the difference in incompatibility group and the fact that the β-lactamase produced is of serotype C rather than A, as is produced by pI258 and pI524 (446). Plasmids of the delta family lack β-lactamase determinants and, like the prototype pII6907 (Table 2), only mediate resistance to inorganic ions (446).

The orphan family consists of plasmids of various incompatibility groups which bear no apparent relationship to the other plasmids on the basis of restriction pattern. A heteroduplex between pI258 and the orphan plasmid pI1071 did reveal homology over approximately one-third of the respective genomes, however; these plasmids are of the same incompatibility group and both encode for cadmium resistance (Cd^r) and elaborate a serotype A β-lactamase (446). A restriction map has been prepared for pSK67, an orphan plasmid which appears similar, if not identical, to pI1071 (Fig. 2; Gillespie and Skurray, unpublished data). The 21.2-kb orphan plasmid pUB101 (Table 2) is unusual in that it does not belong to either incompatibility group 1 or 2, and it encodes resistance to fusidic acid in addition to β-lactamase production and inorganic ion resistance (270, 446).

Evidence for a β-Lactamase Transposon

Although the majority of penicillin-resistant *S. aureus* strains are found to carry β-lactamase plasmids, an increasing number of strains have been described in which the genes for β-lactamase production are apparently located on the chromosome (14, 15, 116, 153, 187, 295, 317, 391, 489, 513). In some instances, the chromosomal β-lactamase determi-

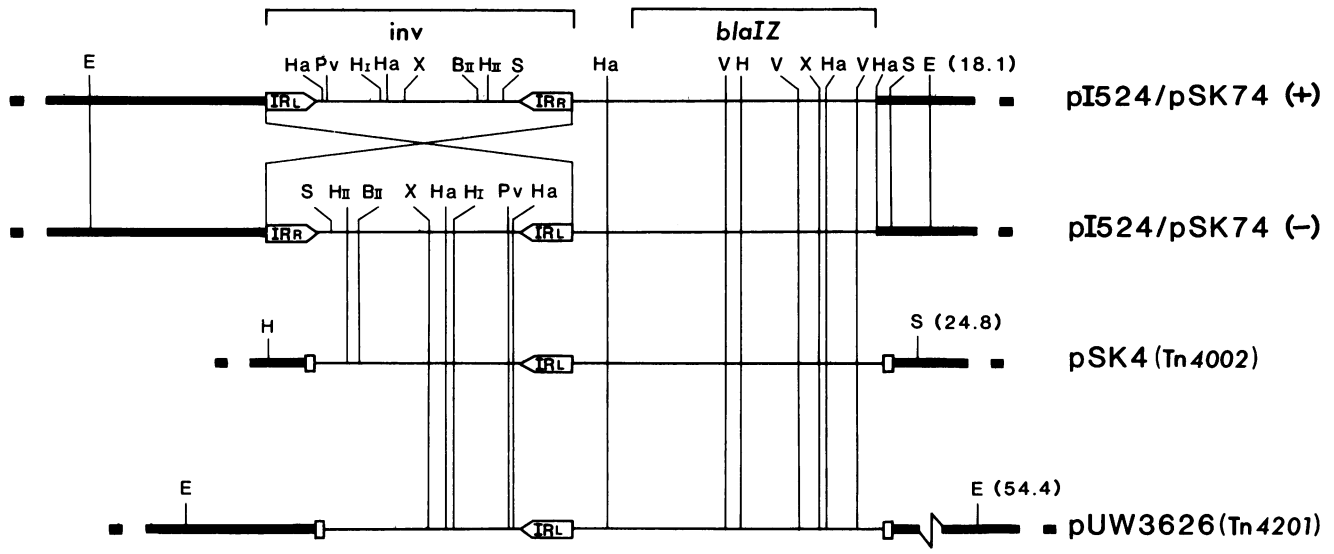

FIG. 3. Restriction maps of the β-lactamase regions of pI524/pSK74, pSK4, and pUW3626 aligned to emphasize identity (Gillespie et al., unpublished data); solid lines represent adjacent sequences on the plasmids. Map coordinates are expressed in kilobases and correspond with those used in the complete maps (see Fig. 2 and 9). Restriction sites are indicated by B_{II} (*Bgl*II), E (*Eco*RI), H (*Hin*dIII), Ha (*Hae*III), H_I (*Hpa*I), H_{II} (*Hpa*II), Pv (*Pvu*II), S (*Sal*I), V (*Eco*RV), and X (*Xba*I). The invertible region (inv) of pI524/pSK74 is shown in the (+) and (−) orientations, and the left-hand and right-hand inverted repeats of inv (IR_L and IR_R, respectively) are designated as for the (+) orientation. The short inverted repeats of the β-lactamase elements in pSK4 (Tn*4002*) and pUW3626 (Tn*4201*) are indicated by the open boxes.

nants were shown to belong to integrated β-lactamase plasmids (221, 441, 556), the sites of insertion of which (Ω 420, Ω 500) have been mapped (Fig. 1) (375). The chromosomal β-lactamase determinant (*bla*) reported by Poston (391), however, was mapped on the chromosome independent of any other plasmid markers (Fig. 1) (375), suggesting that this determinant may be transposable.

Evidence has been presented which suggests that the β-lactamase region of plasmids such as pI524, consisting of one of the inverted repeat sequences, the invertible DNA, and the *blaIZ* loci, may constitute a transposable element, provisionally designated Tn*552* (446). Asheshov (15) first observed that a derivative (pRN4115) of the β-lactamase-negative plasmid pI9789 had acquired, presumably by translocation, a β-lactamase determinant that was normally present on the chromosome of the staphylococcal strain 9789. The β-lactamase encoded by this chromosomal determinant was shown to be very similar to that specified by pI524 (406), and heteroduplex and restriction analyses have revealed that the 6.1-kb inserted β-lactamase region of pRN4115 is in fact indistinguishable from that of pI524 (446). The integration of Tn*552* was presumed to be site specific, as two independent translocations to pI9789 possessed invertible regions at a location apparently identical to that seen in pI524 (332). The possibility exists, however, that the translocation of Tn*552* into pI9789 may not represent transposition, but rather the integration of the β-lactamase region at a specific site adjacent to one of the inverted repeat sequences, a copy of which was suspected to be present on this plasmid (446).

The β-lactamase region of pSK74, a plasmid isolated from Australian strains of *S. aureus* (154) which is equivalent to pI524, was extensively mapped by using a series of restriction endonucleases (Fig. 3; Gillespie and Skurray, unpublished data). This region was found to share substantial restriction identity with a β-lactamase determinant located on the otherwise unrelated 35.1-kb aminoglycoside resistance plasmid pSK4 (see Fig. 3 and 9). Analysis of cloned fragments of these two plasmids revealed that pSK74 and pSK4 are essentially the same with respect to 14 of the 15 restriction sites mapped within the β-lactamase region, but that, unlike pSK74, which has an invertible segment which can be demonstrated in either orientation, the parallel segment in pSK4 appears to be locked in the negative orientation (Fig. 3). It is possible that the β-lactamase region of pSK4 has undergone a deletion of IR_R similar to that recorded for pI13371 (446), which has caused the invertible segment to become locked. Evidence for this comes from the lack of the *Sal*I site which maps near IR_R on pSK74 (Fig. 3) and the absence of a 650-bp inverted duplication in self-annealed molecules of pSK4 which would have been expected to result from homologous pairing of IR_L and IR_R.

Heteroduplexes between pSK4 and the essentially homologous but β-lactamase-negative plasmid pSK1 (Fig. 4) revealed that the 6.7-kb β-lactamase region of the former plasmid is flanked by inverted repeat sequences of approximately 80 bp. β-lactamase elements identical to that present in pSK4 have been demonstrated at various sites and in both orientations in naturally occurring plasmids which are otherwise homologous with pSK1. Inserted derivatives of pSK1 have also been produced in vitro, presumably by transposition of the β-lactamase element from a chromosomal site. To this end, DNA-DNA hybridization has detected the presence of sequences homologous to the *blaIZ* region of pSK4 at a unique site on the chromosomes of such strains, thereby confirming the putative source of the element. From these findings it appears that the 6.7-kb β-lactamase element detected in pSK4 and similar plasmids is a transposable element related, but not identical, to Tn*552*, the β-lactamase transposon detected in pI524 (446). This second transposon has been designated Tn*4002*.

A third β-lactamase transposon, also detected in Australian strains of *S. aureus*, has recently been reported (233). This transposon, designated Tn*3852*, is 7.3 kb in size and has been shown to transpose from the chromosome of a clinical isolate to a plasmid equivalent to pSK1 at high frequency;

however, subsequent transposition from the chromosome of a laboratory strain appeared to be greatly reduced. Despite the difference in reported size, Tn*4002* and Tn*3852* are detected in similar epidemic strains and may prove to be identical.

β-Lactamase production encoded by conjugal Gm^r plasmids isolated in the United States has also been associated with the presence of a characteristic DNA segment (13, 159). Restriction mapping of the β-lactamase region of one of these plasmids, pUW3626 (79), has revealed substantial homology with both Tn*552* and Tn*4002* (Fig. 3; Gillespie and Skurray, unpublished data). The only difference between this region and Tn*4002* appears to be the absence of *Hpa*II and *Bgl*II sites in the inv segment of the former; similar to Tn*4002*, heteroduplexes between pUW3626 and a β-lactamase-negative plasmid exhibited a 6.6-kb structure composed of a single-stranded loop with short inverted repeat sequences. It therefore seems likely that the β-lactamase element from such conjugal plasmids may also be transposable. Conclusive evidence to this effect has been provided by Weber and Goering (D. A. Weber and R. V. Goering, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 701, 1985), who have observed that Pc^r in their plasmids is associated with a 6.6-kb transposon designated Tn*4201*. This transposon is capable of *rec*-independent translocation to plasmid and chromosomal sites, is expressed in either insertion orientation, and can undergo amplification, with consequent elevated resistance, by tandem duplication.

Determination of the exact relationships among Tn*552*, Tn*4002*, Tn*3852*, and Tn*4201* awaits further analysis, as does the possibility that the unique site occupied by Tn*4002* on the chromosome corresponds to the *bla* determinant mapped by Pattee et al. (375) (Fig. 1).

RESISTANCE TO INORGANIC IONS

Inorganic or heavy metal ions such as cadmium, mercury, arsenate, arsenite, antimony, lead, zinc and bismuth are highly toxic to most life forms and are often present in the environment. It is not surprising then, that despite the fact that many of these compounds have not been used therapeutically, resistance has been reported among diverse groups of microorganisms (439, 484, 485, 499). The correlation between heavy-metal resistance and antibiotic resistance among both clinical and environmental isolates is important, however, and should not be overlooked.

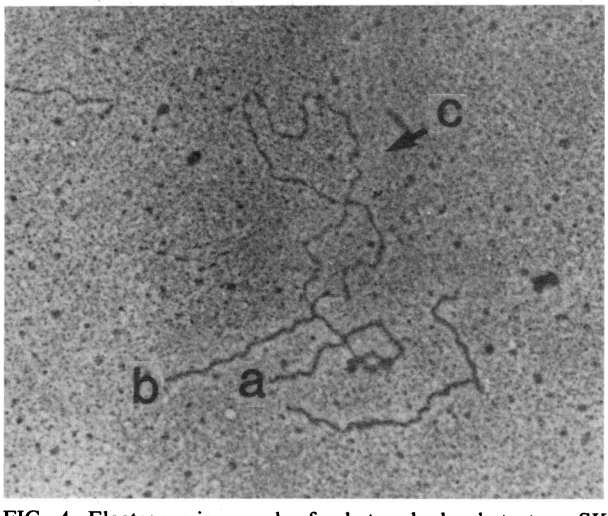

FIG. 4. Electron micrograph of a heteroduplex between pSK4 and the β-lactamase-negative, but essentially homologous, plasmid pSK1 (see Fig. 9 for comparative restriction maps). Plasmid DNA was cleaved with *Eco*RI; the ends of the heteroduplexed fragments are designated a and b. The arrow marked c indicates the single-stranded loop and short double-stranded stem of the 6.7-kb β-lactamase transposon Tn*4002*.

Cadmium Resistance

Cadmium ions enter the bacterial cell via a specific, energy-dependent transport system which normally takes up physiological manganese ions (516). The toxic effect of cadmium is believed to result from the inhibition of respiration caused by the binding of the ions to the sulfhydryl groups of essential proteins (142), although other biological effects are seen (484). *S. aureus* is the only organism in which plasmid-mediated Cd^r has been demonstrated (116, 241, 358), and various studies have revealed that there are at least two distinct mechanisms of Cd^r, encoded by the *cadA* and *cadB* determinants, respectively (381, 465, 517). The gene products of both *cadA* and *cadB* also are considered to confer resistance to zinc ions (381).

The *cadA* determinant causes a 100-fold increase in Cd^r due to a specific efflux system which prevents internal accumulation of cadmium ions (517, 537). Cadmium and possibly zinc ions, but not manganese ions, appear to be extruded from the resistant cells via a mechanism which is energized by the exchange of protons across the membrane (381, 517). Expression of the *cadA* gene is reported to occur constitutively (358, 381). The *cadB* determinant encodes a lower level of Cd^r by a mechanism which does not involve efflux of cadmium ions (381). Experiments with energy-inhibited cells indicated that the *cadB* gene product may be an inducible or amplifiable cadmium and zinc ion binder located in the cell membrane or cytoplasm (381).

The *cadA* determinant constitutes one of the highly conserved regions of β-lactamase plasmids such as pI524, pI258, and pII147 (Fig. 2), and the locus has been accurately mapped on these plasmids by deletion and cloning analysis (288, 355). Plasmid pII147 is notable in that it carries both *cadA* and *cadB* determinants (355, 464). Of interest is the presumed linkage of the genes for resistance to bismuth (*bis*) and lead (*lea*) to *cadA* on pI258 and to *cadB* and probably also *cadA* on pII147 (Fig. 2) (355, 465). The *cadB* gene has also been located on a 3.2-kb Cd^r plasmid, designated pIP983 (Table 2), which shares homology with the *cadB* and mcr region of pII147, and the mcr region of pI258, but is compatible with both plasmids (124). The restriction map of a second 3.2-kb Cd^r plasmid isolated from several strains of phage group II *S. aureus* has been determined (115). Plasmids which were epidemiologically related to pIP983 and contained 40 to 100% of pIP983 sequences have been reported (124). These plasmids ranged up to 34.8 kb in size (e.g., pIP524) and, in addition to Cd^r, encoded resistance to tobramycin, streptogramins, and tetracycline (see Table 4) (126).

In a number of recent clinical isolates of *S. aureus*, Cd^r could not be associated with an extrachromosomal locus (153, 295, 550). While there is no evidence to suggest that Cd^r is transposable, chromosomal Cd^r has been shown in some strains to result from the integration of β-lactamase and heavy-metal resistance plasmids such as pI258 into the chromosome (375, 441). In contrast, the integration of the β-lactamase and heavy-metal resistance plasmid pZA10 (Table 2) into the chromosome led to the inactivation of Cd^r,

thereby implicating the *cad* locus as the integrational recombination site (2).

In recent work, Silver and colleagues (550) were unable to detect homology between a chromosomal low-level Cd^r determinant in methicillin-resistant *S. aureus* and the *cadA* region of pI258. The mechanism of Cd^r in these strains was examined, and although a *cadA*-like energy-dependent efflux system was identified, the parallel resistance to zinc ions mediated by both *cadA* and *cadB* was not observed. These results therefore imply the existence of a third Cd^r determinant which we designate *cadC*. Such a determinant has also been postulated for the low-level Cd^r exhibited by members of the orphan family of β-lactamase plasmids such as pII1106, pII1113, pSK67, and pSK77 (Table 2) (446; Gillespie and Skurray, unpublished data), but it remains to be determined whether the plasmid and chromosomal genes are homologous.

Mercury Resistance

Mercury and organomercurial compounds are toxic to living organisms because of their solubility in lipids and their ability to bind to the sulfhydryl groups of membrane proteins and enzymes (410). Resistance to mercurials is commonly determined by plasmids and may have been selected and spread in the staphylococci as a result of the use of organomercurials, such as phenylmercury and thiomersal, as hospital disinfectants (389) or from the therapeutic use of mercurial diuretics (185); for reviews of resistance to mercurials, see references 410 and 484a. Resistance to inorganic mercury is mediated by the enzyme mercuric reductase which reduces the Hg^{2+} ion to metallic Hg°, a less toxic and highly volatile form (536). Detoxification of organomercurials (Om^r) is mediated by an organomercurial lyase which cleaves the carbon-mercury bonds to produce Hg^{2+} ions that are volatilized in turn by the reductase. Mercuric reductase and organomercurial lyase are coordinately induced by Hg^{2+}, phenylmercuric acetate, and several organomercurials which do not act as substrates (536). This characteristic suggested that the genes for Hg^r (*merA*) and Om^r (*merB*) are under joint regulatory control, and a third gene (*merR*) has been proposed for regulation of the system (536).

The operon nature of the *merA* and *merB* genes was established by analysis of point and transposon insertion mutants of β-lactamase plasmids which also mediated Hg^r Om^r (350, 536). These studies revealed that *merA* is transcribed prior to *merB* and that the regulatory gene(s) probably lies proximal to these determinants. Nucleotide sequencing of a 6.4-kb *Bgl*II fragment encompassing the *mer* region of the β-lactamase plasmid pI258 has identified the *merA* and *merB* genes, which are capable of encoding polypeptides with molecular weights of 58,600 and 23,600, respectively, together with up to six unidentified open reading frames (ORFs) (see Fig. 13) (R. Laddaga, L. Chu, T. Misra, and S. Silver, submitted for publication).

The mercury resistance genes have been mapped adjacent to the *cadA* determinant in a highly conserved region on plasmids pI524, pI258, and pII147 by analyzing deleted variants and cloned fragments (288, 355, 446) (Fig. 2). The observation that spontaneous deletions of an apparently identical 6.5-kb segment of DNA had occurred in Hg^s Om^s derivatives of the alpha family plasmid pUB108 (Table 2) led to speculation that mercury resistance in *S. aureus* may be transposable (446). However, all of the Hg^r resistance plasmids examined, with the exception of pUB101, shared identity in this region, suggesting that independent transposition was not involved in their formation.

As with Cd^r, Hg^r in multiresistant *S. aureus* isolates is often encoded at a chromosomal locus (153, 295, 550) and may either result from a transposon insertion or be due to the chromosomal integration of a heavy-metal resistance plasmid. Sequence identity has been detected between the chromosomes of such Hg^r strains and restriction fragments encompassing the Hg^r-encoding regions of the alpha family plasmid pSK74 and the gamma family plasmid pI258 (550; M. Gillespie, B. Lyon, L. Loo, P. Matthews, P. Stewart, and R. Skurray, submitted for publication). Interestingly, hybridization revealed that the mercury resistance determinant apparently occupied different locations on the chromosomes of some recent isolates of methicillin-resistant *S. aureus* (550), a result which implies the involvement of a transposable element.

Further support for the transposon theory emerges from recent experiments which have shown that the Hg^r operon is flanked by directly repeated sequences of ca. 920 bp (Fig. 2). These direct repeats were initially detected on the pI524-like plasmid pSK74 by comparative restriction mapping and by hybridization with sequences specific to IS*257*, an insertion sequence associated with a plasmid-borne trimethoprim resistance gene (see Fig. 13) (Gillespie et al., submitted; see relevant section). Sequences homologous with IS*257* have also been detected, by hybridization, at unique sites on the chromosome of Hg^r, but not Hg^s, strains of *S. aureus*, and there is clear evidence that these sequences similarly flank the chromosomal *mer* region (Gillespie et al., submitted). On the basis of these results, the Hg^r region on pSK74 has been provisionally designated transposon Tn*4004* (Fig. 13).

Resistance to Arsenate, Arsenite, and Antimony(III)

Arsenate is an analog of phosphate and consequently produces a toxic effect on the bacterial cell by inhibiting enzymes such as kinases and by interrupting energy transfer during glycolysis (for reviews, see references 142, 484, and 485). In *E. coli*, and presumably also *S. aureus*, a relatively indiscriminate phosphate transport system is responsible for bringing arsenate ions into the cell, and arsenate resistance (Asa^r) can result from mutation of the chromosomal gene(s) for this system (458). By far the most common form of Asa^r in both organisms is plasmid mediated, however, and involves reduced uptake of arsenate ions combined with accelerated efflux (458). The mechanism of resistance is thus similar to that for cadmium ions, although efflux of arsenate ions is probably energized by the hydrolysis of adenosine triphosphate rather than proton motive force (459).

The toxicity of both arsenite and antimony(III) is attributed to the capacity of these ions to bind to the cysteine residues of proteins (484). Despite extensive investigations which have revealed that the genetic determinant(s) for arsenite and antimony(III) resistance (Asi^r/Sb^r) is linked to the plasmid-borne Asa^r determinant, the mechanism(s) of Asi^r and Sb^r remains poorly understood (355, 358, 458). It has been shown, however, that Asi^r/Sb^r does not involve the intracellular conversion of the ions to less toxic forms or the excretion by resistant cells of soluble thiols which bind to the ions (458).

Coordinate expression of Asa^r and Asi^r/Sb^r can be induced by any one of the three ions or by bismuth(III), although resistance to the latter is not manifested (458). Genetic evidence that the resistance determinants exist as an "operonlike" structure with a single positive control ele-

ment was provided by Novick and colleagues (355). These authors showed by way of point and deletion mutants that, while the determinants for Asa^r (*asa*) and Asi^r/Sb^r (*asi-ant*) are genetically separable, the genes are controlled by a common promoter and *asa* is transcribed prior to *asi-ant*.

The determinants of Asa^r and Asi^r/Sb^r were originally associated with the β-lactamase plasmids pI524, pI258, and pII147 (358), and subsequent analysis has shown that the region spanning *asa* and *asi-ant* is highly conserved in plasmids of this type (446). The location of the *asa–asi-ant* region on these plasmids (Fig. 2) has been refined by a series of experiments involving recombinational and deletion mapping and cloning of the relevant genes (288, 355). Heteroduplex studies have revealed that the region containing the determinants of Asa^r and Asi^r/Sb^r contains a 2.5-kb sequence flanked by inverted duplications of about 200 bp (446). Although this structure is suggestive of a transposon, no evidence for the transposition of these determinants has been presented.

RESISTANCE TO METHICILLIN AND OTHER β-LACTAMASE-RESISTANT PENICILLINS

The mode of action of the semisynthetic β-lactamase-resistant penicillins and cephalosporins, such as methicillin, cloxacillin, and cephalothin, is similar to that of penicillin G. Enzymes essential for cell wall synthesis, including D-alanine carboxypeptidases and peptidoglycan transpeptidases, covalently bind the penicillin and are thereby inhibited (for reviews, see references 145, 399, 400, 473, and 530). Studies on such PBPs in *S. aureus* have shown that there are four or five distinguishable species with molecular weights of 79,000 to 87,000 (PBP1), 73,000 to 80,000 (PBP2), 70,000 to 75,000 (PBP3), 70,000 (PBP3′), and 41,000 to 46,000 (PBP4) (67, 150, 245, 401, 521, 553); the variations in number and size of PBPs probably reflect the different gel electrophoresis conditions used from laboratory to laboratory rather than major dissimilarities among the different strains used.

Role of PBPs in Methicillin Resistance

Although it is not known which of the PBPs is the lethal target(s) for the penicillins, the isolation of viable mutants which lack PBP1 (97) or PBP4 (98) has been used as an argument that these proteins are nonessential and that it is likely that PBP2 or PBP3 or both are the crucial targets (97). These two proteins appear able to intercompensate their roles, presumably as transpeptidases, although one (PBP3) has been hypothesized to be the primary transpeptidase for the incorporation of newly synthesized peptidoglycan (530, 554, 555).

Resistance to methicillin and other β-lactamase-resistant penicillins could therefore result either from an intrinsic change in one or more PBPs, which provides immunity to all β-lactam antibiotics, or from the synthesis of a new PBP with reduced affinity for β-lactam antibiotics. Whichever, the synthesis of peptidoglycan by a methicillin-resistant (Mc^r) strain of *S. aureus* when grown in the presence of methicillin appeared to occur only in the region of the septum, where cell wall division occurs in actively growing cultures, and not in regions where cell wall thickening was taking place (466). Whereas these results implied that the altered or new PBP in Mc^r strains is a transpeptidase involved in septum formation (539), biochemical studies led Brown and Reynolds (49) to suggest that methicillin resistance is associated with a β-lactam-resistant transpeptidase which functions predominantly in cell wall thickening and perhaps also in septum formation.

There is now a growing body of evidence showing that, in most strains, Mc^r results from the ability of the cell to synthesize a unique PBP. A number of authors have observed a low-affinity satellite protein (PBP2′ or 2a), of molecular weight 74,000 to 78,000, in Mc^r organisms which is absent from the isogenic Mc^s strain (150, 188, 395, 400, 401, 416, 521, 523). Furthermore, re-examination of two Mc^r strains, previously considered to produce an altered PBP3 with a low affinity for β-lactams (48, 190), has demonstrated that this protein is in fact PBP2′ (400–402). Mc^r strains have also been selected in the laboratory by repeated subculture of a Mc^s strain in the presence of increasing concentrations of methicillin, and these apparent multistep mutants produced a low-affinity PBP equivalent in size to PBP2′ found in clinical isolates (500a). PBP2′ is not a derivative of PBP1 or PBP3, and there are conflicting reports which suggest that it may (500a) or may not (400) have evolved from PBP2. PBP2′ is now considered to function as a transpeptidase and to substitute for the activities of one or more essential PBPs in *S. aureus*; PBP2′ remains unsaturated with β-lactams at concentrations of the latter able to kill Mc^s strains (400–402).

Synthesis of PBP2′ is induced upon exposure to β-lactams (70, 416, 521, 523), and it has been suggested that PBPs themselves may play a role in transmitting the necessary regulatory signal across the cell membrane (70). Alternatively, the demonstration that production of PBP2′ is inducible in strains carrying a plasmid encoding an inducible β-lactamase, but constitutive in variants which have lost this plasmid (401, 521), may implicate the product of the β-lactamase repressor gene, *blaI*, in the regulation of PBP2′ synthesis. Mc^r in a strain which lacked β-lactamase activity and was reported to synthesize a high quantity of PBP3 with a low affinity for methicillin (416) may, on further resolution, be shown to result from constitutive PBP2′ synthesis. If, however, PBP3 is implicated, this result, together with findings that in a strain resistant to oral cephalosporins PBP3 was absent or had substantially reduced affinity while PBP2, which was present in an increased amount, bound cephalosporins poorly (150), indicates that intrinsic resistance to β-lactams may not exclusively result from the production of PBP2′.

Strains of methicillin-resistant *S. aureus* exhibit two unusual properties in that the level of resistance to β-lactams is higher at 30°C than at 43°C and also at pH 7.0 versus pH 5.2 (5, 66, 113, 369, 424, 428). Rapid loss of phenotypic resistance with growth at higher temperatures could be correlated with the thermosensitivity of expression of PBPs (48, 190), and the observations that PBP1, PBP2, and PBP2′ are thermolabile (523) and the optimal temperature for induction of PBP2′ is 32°C rather than 37°C (521) augment this contention. Similarly, the molecular basis for the sensitivity at pH 5.2 of organisms that are normally Mc^r may be explained by the absence of PBP2′ and other PBPs in cultures grown under acidic conditions (188, 523).

Intrinsic resistance to β-lactams in clinical strains of *S. aureus* is often heterogeneous in character, being expressed by a minority of cells at 37°C, but uniformly by the entire population in hypertonic media or at 30°C (5, 113, 427, 486). Isolation of the highly resistant minority population on increased concentrations of methicillin produces a population which is once again heterogeneous, but exhibits a higher level of resistance than the population from which it was selected (304). Heterogeneous strains have been shown to possess detectable amounts of PBP2′ irrespective of the

incubation temperature, and it has been suggested that the failure of the entire population to express phenotypic resistance may be explained by a requirement for a second, unidentified factor (189). This factor could control the rate of transcription of the functional gene for PBP2′, modify an initially nonfunctional PBP to be enzymatically active, or regulate autolytic or other irreversible activity until sufficient PBP2′ is available to continue cell wall synthesis (189). The possibility that Mc^r involves control of autolytic enzymatic activity has also been discussed by others (54, 395).

Genetics of Methicillin Resistance

The location of the genetic determinant of Mc^r (*mec*) in *S. aureus* has been the subject of debate despite numerous investigations. The instability of Mc^r in aging cultures (7, 174, 253) lent support to the belief that the determinant was plasmid-borne, and cotransduction and coelimination studies linked Mc^r with plasmid-encoded enterotoxin B production (106, 107). These findings contrasted with those of others who failed to demonstrate the recovery of Mc^s strains following plasmid elimination procedures (81, 227, 479). Moreover, ultraviolet irradiation of transducing phage lysates was shown to enhance the frequency of transduction of Mc^r, a result concomitant with a chromosomal locus (81, 226), and there was no correlation of resistance with the presence of plasmid DNA (227, 263, 479).

The question of the nature of *mec* seemed largely resolved following the transformation of Mc^r by chromosomal, and not plasmid, DNA (460) and the establishment of a chromosomal map location for this determinant (249). The Mc^r determinant was shown by cotransformation to be genetically linked to the novobiocin resistance marker (*nov*) and to mutational sites for purine (*purA*) and histidine (*his*) biosynthesis (Fig. 1). More recently, transposon mutagenesis mapping, using the *S. aureus* Em^r transposon Tn*551*, has revealed a second marker, mediating a 50- to 100-fold increase in Mc^r, which is not physically linked to the *mec* determinant (30, 32). These transposon insertions, designated Ω 2003 and Ω 2004 (Fig. 1), are associated with a number of complex rearrangements and deletions in the region (30). It has been proposed that the two genetically distinct loci may represent the structural gene for PBP2′ and the regulatory gene resonsible for the expression of Mc^r in heterogeneous strains of *S. aureus* (189). Some support for such a proposal has been provided by the cloning, from the chromosome of a Mc^r *S. aureus* strain into *E. coli*, of a determinant which encodes the constitutive rather than the inducible synthesis of a PBP with properties similar to those of PBP2′ (302a). It remains to be demonstrated that the 14.5-kb cloned fragment, which also encodes resistance to the aminoglycoside tobramycin, does indeed correspond to the genetically mapped *mec* region on the *S. aureus* chromosome (Fig. 1).

Analysis of numerous strains of Mc^r *S. aureus* has led some authors to propose that the majority of such organisms have evolved from a single Mc^r clone, characterized by resistance to streptomycin, sulfonamides, and tetracycline and production of a β-lactamase, enterotoxin B, and an orange pigment (223, 227, 255, 263). Resistance to additional antibiotics exhibited by some cultures was thought to have been acquired by transfer of plasmids from other staphylococci (255, 269), and the change of phage-typing pattern among these strains may be the result of an evolutionary sequence of lysogenic events mirrored in Mc^s strains (264, 271). Indeed, probing of clinical Mc^r strains isolated in different countries between 1967 and 1985 revealed a high level of conservation of a 3.5-kb *Bgl*II fragment shown to be associated with *mec* (28). On the other hand, the findings of Cohen and Sweeney (83) with regard to the effect of *mec* on the expression of staphylococcal protein A indicated that there may be genetic differences between *mec* determinants. However, even these authors suggested that the perceived differences could have been the result of genes closely linked to *mec* or the polarity of insertion of *mec* into the *S. aureus* chromosome.

The genetic analysis of *mec* has been further complicated by an apparent requirement for the recipient to be lysogenized with a temperate phage and to carry a β-lactamase plasmid before the transferred determinant could become established (81, 82, 84). Transfer has subsequently been shown to be independent of the lysogenic state of the recipient, but dependent on the presence of either a plasmid or a chromosomal β-lactamase determinant (476). This phenomenon appears to have an in vivo correlate in that most nosocomial isolates of Mc^r *S. aureus* are β-lactamase producers (113, 153, 259). Some workers have suggested that the β-lactamase produced by this determinant may provide limited protection against exposure to methicillin during the initial inefficient period of *mec* expression (476). Others have argued that the presence of β-lactamase plays only a minor role in the recovery of Mc^r transductants (40), although it is interesting that the β-lactamase determinant is reportedly involved in the regulation of expression of PBP2′ (521).

With the evidence of Berger-Bächi and colleagues (28), it now seems plausible that sequences on β-lactamase plasmids other than those associated with production of this inactivating enzyme may be critical for the establishment of *mec*. These authors cloned a 3.5-kb *Bgl*II DNA fragment associated with *mec* which was absent from isogenic Mc^s strains. Hybridization of Mc^r chromosomal DNA with this fragment revealed that the additional DNA in the Mc^r strain could amount to as much as 36.7 kb. The 3.5-kb *Bgl*II fragment was unusual in that it was found to share homology with the sequences flanking the *mer* region on the β-lactamase plasmid pI524 and other large staphylococcal plasmids. This homology is most likely due to the directly repeated IS*257*-like sequences associated with resistance to mercury, which are present on both alpha family β-lactamase/heavy-metal resistance plasmids (see Fig. 2 and 13) and the chromosomes of Mc^r strains (Gillespie et al., submitted). Support for this notion has emerged from the cloning of a 25-kb segment of chromosomal DNA associated with Mc^r in which four copies of a 920-bp repeated sequence were identified (P. R. Matthews, K. C. Reed, and P. R. Stewart, submitted for publication). These directly repeated sequences possess restriction identity and DNA sequence homology to IS*257*. Two of these IS*257*-like repeats bound a structure with physical homology to the Hg^r transposon Tn*4004* (Fig. 13) and two flank an integrated Tc^r plasmid equivalent to pT181 (156; Gillespie et al., submitted). These findings suggest that *mec*, *mer*, and *tet* lie in close proximity on the chromosomes of some strains. Direct evidence for the involvement of *mer*-associated sequences in the establishment of *mec* has been presented by Poston and co-workers (40), who demonstrated that strains of *S. epidermidis* (an Mc^r strain of *S. epidermidis* has been reported to synthesize PBP2′ [521]) which lost chromosomal resistance to mercury and cadmium, presumably by deletion, were no longer effective recipients of *mec*. Together with the evidence outlined above, these data suggest that functions encoded by one or more of the IS*257*-like sequences located in this

region may control or direct the integration of *mec* into the staphylococcal chromosome.

Is Methicillin Resistance Transposable?

The transductional linkage of Mc^r and staphylococcal enterotoxin B production observed by Dornbusch and Hallander (106) has been further investigated and shown to involve a transient association of *mec* with the enterotoxin B determinant and the tetracycline resistance plasmid pSN1 (443). This result, combined with the demonstrated ability of *mec* to integrate into the chromosome independent of the host recombination system (84, 444) and the apparent absence of an allelic equivalent to *mec* in Mc^s cells (477), suggests that the determinant may be capable of "hitchhiking" or transposing between different replicons.

Evidence to this effect has now been presented, with a presumptive "methicillin transposon," designated Tn*4291*, being isolated in a manner similar to that used to detect the Em^r transposon Tn*554* (D. L. Trees and J. J. Iandolo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D84, p. 80, and personal communication). Tn*4291*, of 7.5 kb in size, was captured at a secondary insertion site on plasmid pI524 by blocking the preferred insertion site on the chromosome with another copy of the Mc^r allele, and the subsequent site-specific transposition of the element to the chromosome of an Mc^s strain was demonstrated. Consistent with the proposition that the *mec* region provides or directs unique recombination functions was the observance of an increase in the transposition frequency of Tn*551* in recipient strains carrying the determinant (31).

RESISTANCE TO MACROLIDES, LINCOSAMIDES, AND STREPTOGRAMINS

Macrolides such as erythromycin, oleandomycin, and spiramycin, lincosamides such as lincomycin and clindamycin, and streptogramin type B antibiotics exert a bacteriostatic effect by binding to the 50S ribosomal subunit and arresting protein synthesis (147). The exact step inhibited by these antibiotics, known as the MLS group, is uncertain; however, it is most likely that they block the translocation of peptidyl-transfer ribonucleic acid (RNA) by steric interference or allosteric interaction with the ribosome (109).

Mechanisms of MLS Resistance

Resistance to the MLS group of antibiotics (MLS^r) is caused by a single alteration to the ribosome, namely, the N^6-dimethylation of an adenine residue in the 23S ribosomal RNA (rRNA), which reduces the affinity between the antibiotic and the ribosome (272, 273); for reviews of MLS resistance, see references 109, 532 and 533. The site of action of the methylase has been determined (397, 463), and it appears likely that the decreased binding of antibiotic results from a conformational change to the 23S rRNA rather than methylation of the actual region to which antibiotic is bound (109).

Resistance to streptogramin antibiotics in *S. aureus* can also result from enzymatic inactivation (93). Antibiotics such as pristinamycin and virginiamycin consist of two components, streptogramins A and B, which are synergistic in action. Streptogramin A can be inactivated by an *O*-acetyltransferase (Sg$_A^r$) (103, 278) and streptogramin B can be inactivated by a hydrolase (Sg$_B^r$) (277). The determinants for these enzyme activities (which we designate *sga* and *sgb*, respectively) have been detected together on a series of plasmids isolated in France, of which pIP524 (see Table 4) is the prototype (125, 126, 277, 278). In more recent strains, Sg$_A^r$, presumably due to acetyltransferase inactivation, has been shown to be chromosomally encoded (123).

TABLE 3. Selected *S. aureus* resistance plasmids

Plasmid	Resistance to[a]:	Size (kb)	Reference(s)
pE194	MLS	3.7	195, 359
pE1764	MLS	2.7	211
pE5	MLS	2.7	105
pIP706	MLS	3.4	125
pSK61	MLS	3.9	154
pWG4	MLS Sp (DiP$^+$)[b]	39.0	505
pRJ5	MLS	2.1	26
pC221[c]	Cm	4.6	45, 394
pUB112	Cm	4.1	52
pSK2	Cm	4.5	157, 295, 492a
pSK68	Cm Sm	4.6	154
pC223[c]	Cm	4.6	346
pCW7	Cm	4.2	543
pSK70	Cm	4.6	154
pC194[c]	Cm	2.9	99, 196
pSC194	Cm Sm	7.2	207
pSK54	Cm	5.1	153
pT127	Tc	4.5	359
pT167	Tc		173
pT169	Tc	4.0	102, 209, 287, 348
pT181	Tc	4.4	232, 359
pSK52	Tc	4.4	154
pSN1	Tc	4.2	445
pTP-5	Tc	4.5	342
pRC701	Tc	4.0	89
pSK89	Eb Qa	2.4	Gillespie and Skurray, unpublished data
pWG32	Eb Qa	2.4	130

[a] See Table 1, footnote *b*, for abbreviations of antimicrobial agents.
[b] DiP$^+$, Diffusible pigment production.
[c] Members of the pC221, pC223, and pC194 families of Cm^r plasmids are listed below each prototype.

Reports of MLS resistance in *S. aureus* have often referred to an apparent "antagonism" between erythromycin and other MLS antibiotics, and strains were said to exhibit "dissociated" or "generalized" resistance (148, 533). The latter two terms were used to describe organisms which are now known to be either inducibly or constitutively resistant to the MLS antibiotics, respectively, and share a common mechanism of resistance via methylation (533). Strains demonstrating the dissociated phenotype are resistant to erythromycin and oleandomycin, but sensitive to the other MLS antibiotics. The presence of erythromycin or oleandomycin, however, will induce resistance to all of the MLS group, hence, the observed antagonism. Strains demonstrating the generalized or "double" phenotype are constitutively resistant to all of the MLS antibiotics.

Plasmid-Encoded Inducible MLS^r

Inducible resistance to the MLS antibiotics in *S. aureus* is commonly mediated by small multicopy plasmids, the prototype of which is pE194 (Table 3). Plasmid pE194 was originally described by Iordănescu (208) and has been extensively studied in both the natural host (211, 534) and *B. subtilis*, where it was found to replicate and express resistance (180, 195, 456, 457). This plasmid is 3.7 kb in size, is usually present in the cell with a copy number of 10 to 25,

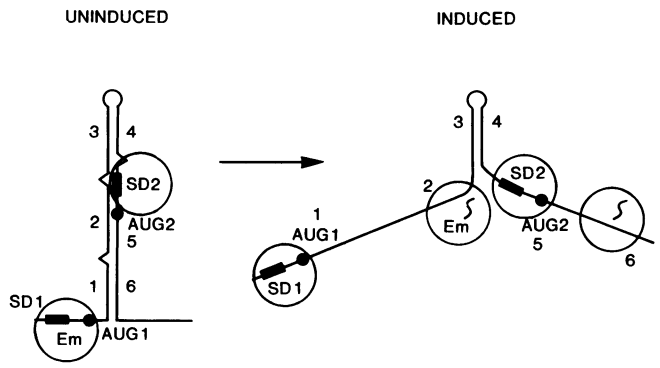

FIG. 5. Translational attenuation model for induction of MLS resistance (adapted from reference 109). Illustrated are the predicted secondary structures for the leader sequence of the *ermC* transcript in uninduced and induced formats. The closed boxes represent ribosome-binding sites (Shine-Dalgarno [SD] sequences), the small circles are the translational start signals AUG1 and AUG2, and the large circles represent ribosomes. In the uninduced format, the ribosome-binding site for the 19-amino acid peptide (SD1) is accessible, whereas that for the rRNA methylase (SD2) is sequestered by base pairing. Translation of the 19-amino acid peptide begins at AUG1, but is stalled in the presence of erythromycin at segment 2. Conformation of the mRNA is thereby altered to the induced format, exposing SD2 and allowing the translation of the methylase from AUG2 by other uninhibited ribosomes.

and belongs to incompatibility group IncI1 (211, 534). A second MLS^r plasmid, designated pE1764 and 2.7 kb in size, belongs to Inc group 12 (211). It has been demonstrated that, while other MLS^r plasmids (e.g., pE5, pSK61; Table 3) may possess resistance determinants which are homologous with pE194, the replication regions of such plasmids do not share homology with pE194 (105; Gillespie and Skurray, unpublished data).

The nucleotide sequence of pE194 contains 3,728 bp and exhibits six ORFs coding for putative polypeptides with molecular weights of 48,300 (A), 29,200 (B), 14,000 (C), 13,900 (D), 12,600 (E), and 2,700 (F) (195). Although six polypeptides have been detected in *B. subtilis* minicells containing a high copy number mutant of pE194 (457), only two of these proteins, of molecular weights 54,000 and 29,000, can be related to the ORFs (195). Polypeptide A would seem to be dispensable for both plasmid replication and MLS^r, while polypeptide B is equivalent in size to the previously characterized methylase encoded by the *ermC* gene of pE194 (455, 457).

Analysis of the nucleotide sequence of *ermC* has led to a proposal for a translational attenuation model for regulation of the MLS^r phenotype (179, 194). The *ermC* messenger RNA (mRNA) was shown to possess a 140-bp leader sequence containing a short ORF capable of encoding a 19-amino acid peptide preceded by a ribosome-binding site, the Shine-Dalgarno sequence SD1 in Fig. 5. Base pairing of the leader sequence can potentially sequester the ribosome-binding site (SD2) for methylase synthesis; however, erythromycin-induced stalling of a ribosome in the act of translating the 19-amino acid peptide is hypothesized to open the folded mRNA, thereby allowing access of a second ribosome to the SD2 binding site and permitting translation of the methylase-coding sequences (Fig. 5). Considerable support for this induction model has been provided through research in the laboratories of Dubnau and Weisblum, as reported in two recent reviews (109, 533). More recently, ribosome-binding studies and analysis of the RNA structures of the leader region have demonstrated that, in uninduced cells, SD2 is not available for ribosome binding, whereas SD1 is (340); there is also firm evidence that the leader peptide is translated (305) and that this translation is required for induction (110), as the model predicted.

Chromosomally Encoded Inducible MLS^r: Role of Tn*554*

Inducible resistance to the MLS antibiotics can also be encoded at a chromosomal locus in clinical isolates of *S. aureus* (259). Such strains often shared additional resistance to the aminocyclitol spectinomycin (Sp^r), and this double resistance phenotype has, in some isolates, been demonstrated to be encoded on the 6.7-kb transposon Tn*554* (247, 327, 384). Tn*554* is unusual among transposons in that it does not possess inverted or direct repeat sequences at its termini (Fig. 6) and does not generate target duplications upon transposition (331, 334). This transposon also exhibits a high preference for a single site in the *S. aureus* chromosome (Fig. 1) (384), always inserting between the same nucleotide pair and in the same orientation (247, 331). When this site is cloned in a plasmid vector, the transposon loses its orientation specificity but retains its site specificity, such that insertion occurs on one side or the other of a 6-bp "target sequence" depending on the orientation (331). Interestingly, this 6-bp sequence matches the terminal 6 bp of the right end of the transposon; however, it is unlikely to account for the insertional specificity of Tn*554*, as a sequence of this size was estimated to occur approximately 1,000 times in the staphylococcal chromosome (331).

Transposition of Tn*554* to secondary sites in plasmids or phage can be obtained, at 100- to 1,000-fold-lower frequency than insertions into the primary chromosomal target, if the latter is occupied by a suitably marked copy of the element (247, 334). The reduction in transpositional frequency under these conditions is believed to be caused by the "transposition inhibition" sequence (*tnpI*) located to the leftmost 89 bp on the resident Tn*554* (Fig. 6) (327). The plasmids which carried insertions of Tn*554* varied considerably with respect to their ability to act as transposon donors, suggesting that the nature of the donor flanking sequences may influence the frequency of transposition (334). Transposition of Tn*554* from such plasmids most often occurred by a duplicative process. Tn*554* has also been transduced to the recipient independent of the plasmid carrier. In this situation, it was believed that Tn*554* inserted into the transducing phage genome and subsequently transposed to the chromosome, a procedure referred to as hitchhiking (334, 352). More recent work, however, provides evidence against a transient, or otherwise, transposition of Tn*554* into the phage DNA (R. Novick and I. Edelman, unpublished data communicated by E. Murphy).

The complete nucleotide sequence of Tn*554* has been reported (330). The transposon is 6,691 bp in length and contains six reading frames of >125 amino acids (Fig. 6). Characterization of insertion and deletion mutants has revealed that three of the ORFs, designated *tnpA*, *tnpB*, and *tnpC*, are essential for transposition of Tn*554*. A fourth reading frame, referred to as *spc*, encodes an aminoglycoside adenyltransferase [AAD(9)], of molecular weight 28,943, which mediates resistance to spectinomycin (329). The sequence of *ermA*, the MLS^r determinant of Tn*554*, predicts a protein of 28,380 molecular weight which is homologous to the rRNA methylase specified by *ermC* (328). The *ermA* transcript also possess a 211-bp leader that

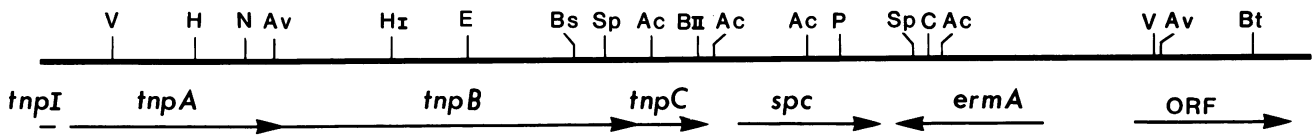

FIG. 6. Functional map of Tn*554* determined by nucleotide sequencing and deletion mutagenesis at the designated restriction sites (330). Restriction sites are indicated by Ac (*Acc*I), Av (*Ava*I), B_{II} (*Bgl*II), Bs (*Bst*EII), Bt (*Bst*NI), C (*Cla*I), E (*Eco*RI), H (*Hin*dIII), H_I (*Hpa*I), N (*Nde*I), P (*Pst*I), Sp (*Sph*I), and V (*Eco*RV). The directions of transcription of the six potential reading frames are indicated. Three reading frames (*tnpA*, *tnpB*, and *tnpC*) encode functions required for transposition of Tn*554*, *spc* encodes an AAD(9), *ermA* encodes the rRNA methylase, and ORF is of unknown (if any) function. The locus responsible for *trans* inhibition of Tn*554* transposition (*tnpI*) is also indicated.

potentially encodes a 19-amino acid peptide which has 13 amino acids in common with the leader peptide of *ermC*. These findings, along with an analysis of the secondary structures of mRNA, indicated that, as with *ermC*, the expression of *ermA* may be regulated by translational attenuation (328).

The possible existence of a Tn*554*-like element on a plasmid which produced a diffusible pigment (DiP^+) has been reported (511). The 39-kb plasmid pWG4 (Table 3) mediated inducible MLS^r together with Sp^r, and transduction of this plasmid resulted in duplication of the MLS^r Sp^r determinants on the chromosome of the recipient. Recent strains of multiresistant *S. aureus* from Australian and London hospitals most frequently exhibit inducible, chromosomal resistance to erythromycin (154, 293, 295, 503, 513), and it has been suggested that this, and the accompanying Sp^r, may be encoded by a transposon such as Tn*554* (154, 157). Such a notion is supported by the demonstration of transposition of Em^r from the chromosomes of Em^r Sp^r isolates from a U.K. hospital to a β-lactamase plasmid (259). There are, however, reports of chromosomally encoded inducible Em^r in strains which are Sp^s (259) and such may result from the chromosomal integration of a plasmid equivalent to pE194; integration of pE194 into the *B. subtilis* chromosome has been achieved in the laboratory (191).

Constitutive MLS^r: Role of Tn*551*

Constitutive resistance to the MLS antibiotics in *S. aureus* has been reported among recent isolates from Brazil (26), France (493), and the United Kingdom (259). In the strain from Brazil, constitutive MLS^r was encoded by the 2.1-kb plasmid pRJ5 (Table 3), whereas the absence of plasmid DNA in the U.K. isolates indicates a chromosomal locus; the nature of encodement of constitutive MLS^r was not reported for the strains from France.

The most thoroughly studied constitutive MLS^r determinant is that carried by a set of epidemiologically related plasmids isolated in Japan in the early 1960s (322). The prototype of this set is the 28.2-kb gamma family β-lactamase and heavy-metal ion resistance plasmid pI258 (Table 2, Fig. 2) (355). The MLS^r determinant (*ermB*) of pI258 has been shown by genetic and molecular analysis to be carried by a 5.3-kb transposable element designated Tn*551* (350). Tn*551* can undergo transposition to multiple plasmid and chromosomal sites independent of the host recombination system (349, 350), and the transposon has consequently become an important tool for mutagenesis studies of staphylococcal plasmid (10) and chromosomal (30, 292, 372, 398) genes. Nucleotide sequencing of the ends of Tn*551* has revealed that the transposon possesses 40-bp inverted terminal repeats and that, upon transposition, this element generates 5-bp direct repeat sequences at the target site (231).

It should be noted that a single point mutation in the attenuator region of the inducible *ermC* methylase can lead to constitutive expression of MLS^r by cells carrying pE194 (532). Presumably, similar mutations were responsible for the constitutive mutants obtained in vitro from inducible strains (47, 265) and, as discussed by Weisblum (533), may also account for the emergence of constitutive MLS^r in vivo such as during treatment of Em^r staphylococci with the lincosamide clindamycin (265).

RESISTANCE TO CHLORAMPHENICOL

Chloramphenicol causes a bacteriostatic effect by binding to the 50S ribosomal subunit and inhibiting the transpeptidation step in protein synthesis (452). Resistance to chloramphenicol in *S. aureus* is due to detoxification; the antibiotic is acetylated by acetyl coenzyme A through the activity of an inducible enzyme, chloramphenicol acetyltransferase (CAT), of which there are at least five variants in *S. aureus* (431, 453). Each CAT is a tetramer composed of identical subunits of approximately 25,000 daltons, and amino acid sequence data for the subunits reveal that the various CATs are closely related (139, 453) (see references 452 and 453 for recent reviews of Cm^r).

Chloramphenicol Resistance Plasmids

Chloramphenicol resistance in *S. aureus* is exclusively mediated by a group of multicopy plasmids which range in size from 2.9 to 5.1 kb (Table 3). A study of incompatibility showed that the Cm^r plasmids pC221, pUB112, pC223, and pC194 each belong to a different incompatibility group, suggesting that their origins may be diverse (212). This premise is supported by the considerable variability in the properties of the protein-DNA relaxation complexes formed by these plasmids (346). Restriction mapping of several Cm^r plasmids has also demonstrated differences in their molecular structure (52, 492a, 542, 543). However, DNA-DNA hybridization and restriction endonuclease analyses have shown that Cm^r plasmids can be grouped into at least three families, with pC221, pC223, and pC194 being the prototypes (Table 3; Gillespie and Skurray, unpublished data). Although Cm^r plasmids from the different families exhibited substantial structural variability, members within each family demonstrated identity over the region known to encode the CAT, and in the cases of the pC221 and pC194 families, regions associated with replication were also found to be homologous (Fig. 7). pC221 encodes for a type C CAT, whereas pC194 encodes a CAT of electrophoretic mobility between type B and C enzymes (452); the type of CAT encoded by pC223 has not been reported, although the CAT-coding

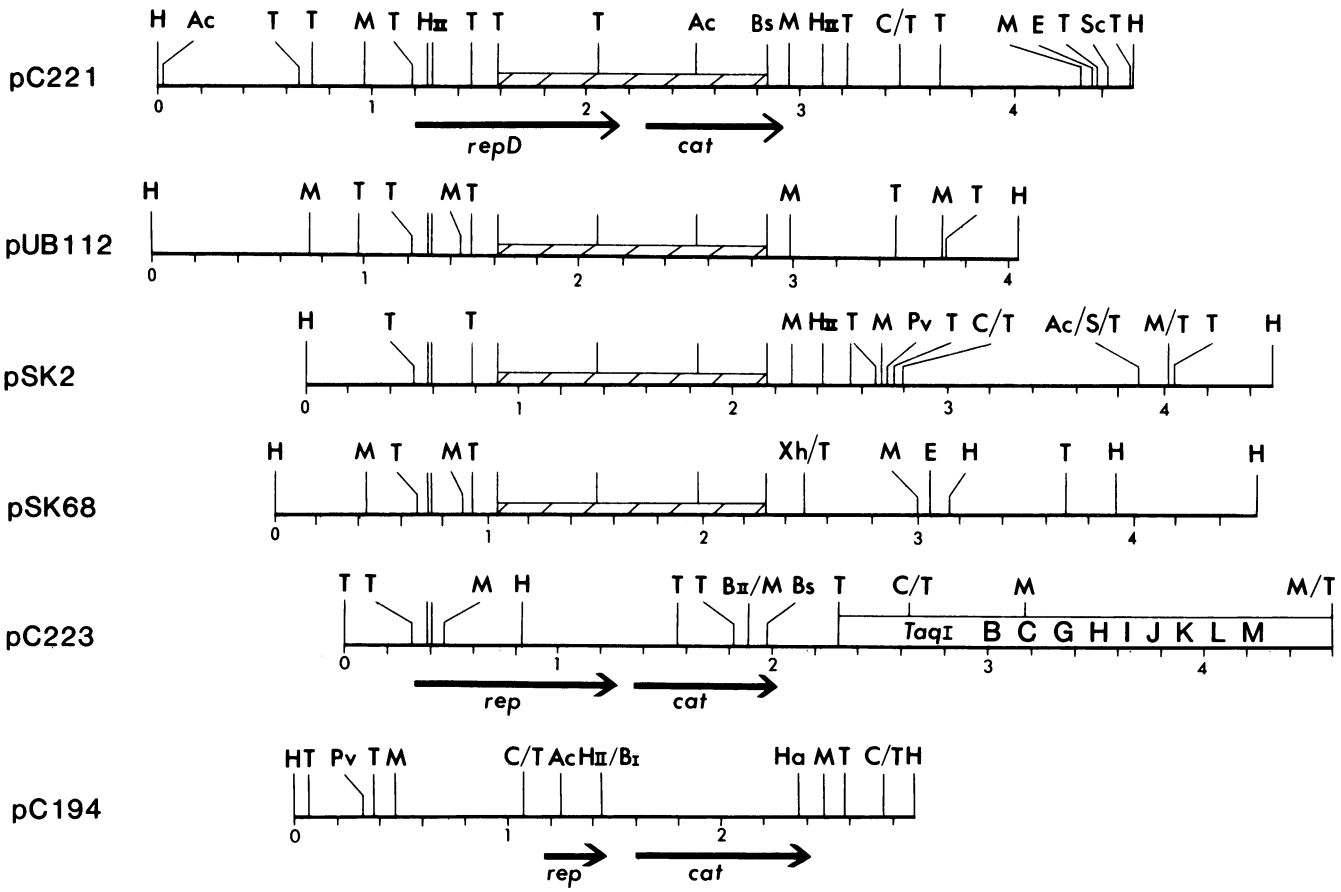

FIG. 7. Linear restriction maps of the chloramphenicol resistance plasmids pC221, pUB112, pSK2, pSK68, pC223, and pC194 (45, 196, 394, 492a; Gillespie and Skurray, unpublished data); distance scales (kilobases) are shown below each map. Restriction sites are Ac (*Acc*I), B_I (*Bgl*I), B_{II} (*Bgl*II), Bs (*Bst*EII), C (*Cla*I), E (*Eco*RI), H (*Hin*dIII), H_{II} (*Hpa*II), Ha (*Hae*III), M (*Mbo*I), Pv (*Pvu*II), S (*Sal*I), Sc (*Sac*I), T (*Taq*I), and Xh (*Xho*I). Nine unordered *Taq*I fragments (B, C, G, H, I, J, K, L, M) are indicated on pC223. Restriction maps of the pC221 plasmid family (pC221, pUB112, pSK2, and pSK68) are aligned relative to an area of common identity indicated by the hatched lines. The regions of pC221 which encode CAT (*cat*) and replication functions (*repD*) are indicated below the map (45, 394); the *cat* and replication (*rep*) regions of pC223 (Gillespie and Skurray, unpublished data) and pC194 (195) are similarly shown. Arrows indicate the directions of transcription of these genes.

region showed some degree of homology with the pC221 CAT sequence (Gillespie and Skurray, unpublished data).

The nucleotide sequence of pC194 has been determined and indicates that the 2,906/2,910-bp plasmid possesses four ORFs which are theoretically capable of encoding proteins with molecular weights of 27,800 (A), 26,200 (B), 15,000 (C), and 9,600 (D) (99, 196). Interruption and deletion analysis revealed that frame B encodes the CAT structural (*cat*) gene while frame C contains sequences associated with plasmid replication (99, 196); the frame D product is involved in plasmid maintenance (1). Sequencing of pC221 has shown that this plasmid also contains four ORFs in a total size of 4,555/4,557 bp (45, 394). The smallest reading frame encodes a CAT of 25,900 molecular weight, which shares 118 amino acid residues in common with the CAT encoded by pC194 (454). The second largest reading frame encodes *repD*, a 37,800-molecular-weight protein associated with plasmid replication. The CAT gene of pUB112 has also recently been sequenced and was shown to encode a protein that differed from the CAT of pC221 by only four amino acids (51), a result consistent with this plasmid being allotted to the pC221 family.

Induction of Chloramphenicol Resistance

Nucleotide sequencing of the genes for CAT has contributed to an understanding of the molecular mechanisms involved in the induction of Cm^r. The presence of an inverted repeat structure preceding the CAT-coding region is believed to result in the formation of a stable stem-loop in the mRNA which sequesters the CAT translation initiation sequence (4, 45, 62). A translation attenuation model similar to that which operates in the induction of *ermC* has been proposed for the induction of the CAT gene of pUB112 (51) and could also be applicable to the CAT of pC221 since these plasmids share strong sequence homology in the region preceding the CAT ORF (51). Thus, ribosome stalling caused by chloramphenicol during translation of the mRNA leader, which encodes a nine-amino acid peptide, opens the stem-loop and enables CAT synthesis to proceed. An alternative hypothesis for induction of the pC194 CAT gene is required, however, as no potential leader peptide sequence was detected (62). It was therefore proposed that the CAT translation initiation sequence is unmasked by the binding of the 5′ part of the stem to complementary sequences in 23S rRNA (62). These rRNA sequences become available for

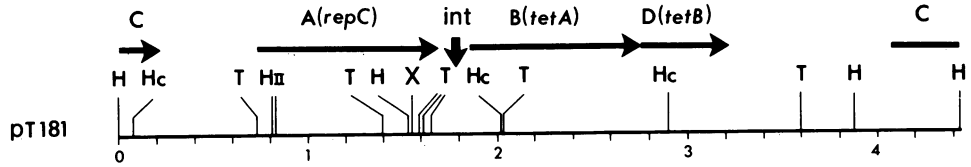

FIG. 8. Simplified restriction map of pT181 taken from Khan and Novick (232). Restriction sites are indicated by H (*Hin*dIII), H_{II} (*Hpa*II), Hc (*Hin*cII), T (*Taq*I), and X (*Xba*I); a distance scale (kilobases) is shown below. The regions which encode the four ORFs are designated A (*repC*), B (*tetA*), C, and D (*tetB*), with the arrows indicating the direction of transcription (232, 356). The arrow marked int denotes the site of integration of the plasmid with the staphylococcal chromosome (156).

binding as a result of interactions between the 50S ribosomal subunit and the chloramphenicol inducer.

RESISTANCE TO THE TETRACYCLINES

Members of the tetracycline family of antibiotics are actively transported into the bacterial cell by a process which is energized by proton motive force (314). The attachment of these antibiotics to the 30S ribosomal subunit sterically interferes with the binding of aminoacyl-transfer RNA molecules to the ribosomal acceptor site. The resultant inhibition of protein synthesis causes a bacteriostatic effect (283).

Tetracycline resistance is associated with a decrease in the intracellular accumulation of the antibiotic (73), and, in *S. aureus*, this was originally correlated with decreased uptake (248, 468). It now seems more likely, however, that staphylococcal resistance is due to a specific efflux mechanism similar to that demonstrated in strains of Tc^r *E. coli* (315). Two major phenotypic patterns of Tc^r have been observed in *S. aureus* (16, 74). The first is an inducible resistance to tetracycline but not to the semisynthetic tetracycline minocycline, mediated by small plasmids, while the second is a constitutive resistance to both tetracycline and minocycline encoded by a chromosomal determinant; for reviews of Tc^r, see references 142 and 283.

Tetracycline Resistance Plasmids

Tetracycline resistance in *S. aureus* is commonly mediated by one of a family of closely related plasmids which belong to the Inc3 incompatibility group (212). These plasmids range in size from 4.0 to 4.5 kb and are multicopy, being present in 20 to 50 copies per cell (Table 3). Detailed restriction endonuclease maps of several Tc^r plasmids exist (342, 347, 445), and the nucleotide sequence of one, pT181, has been determined (232). This plasmid, of 4,437 bp, contains four ORFs which code for putative polypeptides with molecular weights of 37,500 (A), 35,000 (B), 23,000 (C), and 18,000 (D) (Fig. 8). Of these, polypeptide A has been detected in cell extracts (230), purified (240), and shown to correspond to the *repC* gene product which is required for replication of pT181 (347), whereas polypeptide B (*tetA*) and possibly polypeptide D (*tetB*) are involved in mediating Tc^r; *tetA* and *tetB* reading frames overlap and would be expected to be transcribed from a common promoter in the direction shown in Fig. 8 (232, 356).

The replication of pT181 has been studied in some detail by Novick and colleagues (356). Replication is initiated by the *trans*-acting *repC* product and proceeds unidirectionally, and in the same direction as transcription of *repC*, from an origin which has been located in a 127-bp region within the promoter-proximal portion of the *repC*-coding sequence (69, 229, 347). The *repC* protein exhibits sequence-specific endonuclease and topoisomeraselike activities, and a nick site detected within the origin region probably corresponds to the start site of pT181 replication (240). pT181 replication is negatively controlled by two short RNA species, transcribed from within the *repC* leader sequence, that are believed to act by interfering with *repC* translation (250). Comparison of the *repC* polypeptide with the *repD* polypeptide of pC221 revealed that the two initiator proteins share 81% identity at the amino acid level, but diverge significantly in the regions associated with recognition of the respective plasmid origins (45, 394).

With a molecular weight of 35,000, the size of the pT181 polypeptide B approximates that of a 32,000-molecular-weight membrane protein shown to be associated with both plasmid- and chromosome-mediated Tc^r in *S. aureus* (467). The sequence of polypeptide B also predicts an abundance of hydrophobic amino acids, a finding which would be expected for a membrane protein (232). Analysis of the leader sequence which precedes the start site for polypeptide B (*tetA*) revealed the presence of a potential reading frame for a 16-amino acid peptide, together with repeat sequences capable of forming stem-loop structures (232). The presence of these structures led Khan and Novick (232) to suggest that the induction of Tc^r occurs by a translational attenuation mechanism similar to MLS^r induction.

Chromosomal Tetracycline Resistance Determinants

Chromosomal resistance to tetracycline was first reported in methicillin-resistant strains of *S. aureus* (226) and has subsequently been observed in numerous clinical isolates (16, 74, 154, 295, 436, 548). A genetic determinant (*tet-3490*) which mediates resistance to tetracycline but not to minocycline has been mapped on the *S. aureus* chromosome between the Mc^r determinant (*mec*) and the determinant for resistance to fusidic acid (*fusA*) (474, 475) (Fig. 1). Independent analysis of such chromosomal Tc^r determinants by two groups has revealed the presence of an integrated Tc^r plasmid with considerable homology, if not identity, to pT181 (156; J. Jones and P. Pattee, personal communication). Integration of this plasmid appears to have occurred at a unique site, adjacent to a region associated with *mec* (Matthews et al., submitted), in the chromosomes of all strains examined to date (156; Gillespie et al., submitted). The site of integration (int) on the plasmid has been determined by relating hybridization data to a map of pT181 and was concluded to lie within a 155-bp region located between the C-terminal sequences of *repC* and the *tet* promoter (156) (Fig. 8); sequence analysis of the integrated Tc^r plasmid has confirmed this conclusion (A. Elizur-Chandler, P. Matthews, and P. Stewart, personal communication).

A second chromosomal determinant, which is sometimes encountered in conjunction with plasmid or "integrated plasmid" type Tc^r (16, 156), expresses constitutive resist-

ance to both tetracycline and minocycline. This determinant has been designated *tmn* and is located on the chromosome near genes for riboflavine (*rib*) and purine (*purB*) biosyntheses (Fig. 1) (471, 436). The *tet-3490* and *tmn* determinants are thus quite distinct in their chromosomal location, although little is known about the possible genetic relatedness of the two or even if they mediate Tc^r via a similar mechanism.

RESISTANCE TO THE AMINOGLYCOSIDES

The aminoglycoside antibiotics are a group of clinically important compounds which are perhaps more definitively termed aminoglycoside-aminocyclitols since they are differentiated from other related substances through the possession of an aminocyclitol unit. The aminoglycosides can be divided on the basis of whether the aminocyclitol unit is streptidine or 2-deoxystreptamine. The best known streptidine-containing aminoglycoside is streptomycin, while 2-deoxystreptamine-containing aminoglycosides include two subgroups characterized by substitution of the 2-deoxystreptamine unit at positions 4 and 5 (butirosin, neomycin, and paromomycin) or positions 4 and 6 (amikacin, gentamicin, kanamycin, netilmicin, sisomicin, and tobramycin) (56, 383).

Aminoglycosides are taken into the bacterial cell in three stages, viz., (i) energy-independent binding to the outer surface components of the cell; (ii) energy-dependent (phase I) transport across the cytoplasmic membrane, in which the positively charged antibiotic is considered to bind to a specific membrane "transporter" and together these are "driven" or "pulled" across the cytoplasmic membrane by virtue of the cell's internal negative charge; and (iii) a second, more rapid, energy-dependent (phase II) uptake after ribosome binding and perhaps coincident with the lethal event (56, 57, 186, 321). The involvement of a membrane transporter system in aminoglycoside entry has, however, been disputed, with some authors proposing that uptake is due solely to the difference in electrical potential across the membrane (100).

Once inside the cell, the aminoglycoside antibiotics bind to proteins of the 30S ribosomal subunit and thereby inhibit protein synthesis; misreading of mRNA codons has been demonstrated at subinhibitory levels (147, 163). The exact mechanism by which aminoglycosides produce their lethal effect is not clear and has been ascribed variously to the block in protein synthesis, to misreading which would generate faulty proteins, or to disorganization of the cytoplasmic membrane during the energy-dependent transport phase (56, 147, 163, 186); these three events have been incorporated into a recently proposed model for the bactericidal action of aminoglycosides (101a). Miller and co-workers (121, 301, 302) have shown that the lethal effect of gentamicin in *S. aureus* is proportional to the magnitude of the electrical potential across the membrane and that a critical threshold potential is required to initiate uptake of this antibiotic.

Mechanisms of Aminoglycoside Resistance

Bacterial resistance to the aminoglycosides can result from three distinct mechanisms (for reviews, see references 56, 142, 383, and 447). Mutations in the genes encoding ribosomal proteins can lead to alterations in the structure of the ribosome such that it no longer binds the antibiotic. This mechanism has been identified in clinical isolates of streptomycin-resistant *S. aureus* (260), but does not appear to be a significant form of resistance to 2-deoxystreptamine aminoglycosides (383). A second mechanism, which provides low-level cross-resistance to most aminoglycosides and is often referred to as impermeability, results from mutations which affect the energization of the membrane and thereby diminish the uptake of the aminoglycoside (321, 383). The third and most widespread mechanism of resistance to the aminoglycosides is the modification of the antibiotic by cellular enzymes such as aminoglycoside acetyltransferases (AAC), AAD, or aminoglycoside phosphotransferases (APH).

Aminoglycosides modified at the amino groups (by AACs) or hydroxyl groups (by AADs or APHs) no longer bind to ribosomes in vitro and therefore do not inhibit protein synthesis (557). Since modification is not an extracellular process but is considered to occur during the transport of the antibiotic across the cytoplasmic membrane, with which the modifying enzyme is associated (56), resistance may result from a failure in transport (101) or simply from accumulation of the modified, inactive form of the antibiotic at a greater rate than the active form (see reference 56). Evidence suggests that different mechanisms may operate for different aminoglycosides and perhaps in different genera (56, 383); in *S. aureus*, modification did not affect uptake of gentamicin when used at high concentration, but at lower concentrations uptake was reduced compared with that in sensitive organisms (298).

Streptomycin Resistance

Clinical isolates of *S. aureus* commonly exhibit chromosomal resistance to streptomycin (87, 125, 153, 227, 295). Resistance in many "early" strains was of the high-level type, with minimum inhibitory concentrations (MICs) of >10 mg/ml (175, 255), which in all characterized instances was shown to be due to chromosomal mutations (*strA*) affecting the ribosome (260). Low-level Sm^r (MICs, <100 μg/ml) has usually been associated with plasmid-mediated enzymatic modification of the aminoglycoside (172, 255, 383); however, recent isolates from Australia have demonstrated low-level chromosomal resistance (513), and we have tentatively designated this determinant *strB*. APH(3″) (383) and AAD(6′) (487) activities, which modify streptomycin, and an AAD(3″)(9) activity, which modifies streptomycin, together with the pure aminocyclitol spectinomycin (92), have been detected in *S. aureus*.

Unstable resistance to streptomycin is indicative of a plasmid locus (172, 177, 324), and several small multicopy Sm^r plasmids have been isolated (Table 4). Such plasmids are usually present in about 20 copies per cell and have been shown to belong to the Inc5 incompatibility group (212, 359). Examples of Sm^r plasmids (pSC194, pSK68) which also mediate chloramphenicol resistance and are presumably cointegrates between small Sm^r and Cm^r plasmids have been detected (Table 3) (154, 207). Large streptomycin resistance plasmids such as the 45- to 51-kb pWG14 and the 17.55-kb pWA1 have also been identified (Table 4). These plasmids are interesting in that they mediate resistance to a number of other antibiotics such as neomycin, kanamycin, lincomycin, erythromycin, spectinomycin, and fusidic acid; however, they appear to be quite rare (6, 265). Plasmid pWA1 has been shown to mediate Sm^r Sp^r via an AAD(3″)(9) which was also expressed when the plasmid was cloned in *E. coli* (92).

TABLE 4. *S. aureus* aminoglycoside resistance plasmids

Plasmid	Resistance to[a]:	Aminoglycoside-modifying enzymes	Size (kb)	Reference(s)
pS177	Sm	ND[b]	4.35	359
pS194	Sm	ND	4.5	207
pWG14	Sm Nm Km Em Sp (DiP$^+$)[c]	ND	45–51	502
pWA1	Sm Nm Km Sp Fa	AAD(3″)(9) APH(3′)III	17.55	92, 265
pSH2	Nm Km	APH(3′)III	15.15	92, 481
RN1956	Nm Km	APH(3′)III	15.9	92
pTU512	Km Pc Em	APH(3′)III	44.7	244, 520
pTC128	Nm Km Tc Tp Eb	ND	36	87
pUB110	Nm Km	AAD(4′)(4″)	4.5	36, 72, 303, 311
pAP010	Nm Km Tm	AAD(4′)(4″)	4.2	279
pSK50	Nm Km Tm	ND	4.5	154
pIP524	Tm Sg_A Sg_B Cd	AAD(4′)(4″)	34.8	126, 279
pIP630	Tm Sg_A Sg_B Cd Tc	ND	26.1	126, 279
pIP629	Tm Sg_A Sg_B Tc	ND	29.55	126, 279
RPAL	Gm Tm Km	AAC(6′) APH(2″)		280, 470
pSK41	Gm Tm Km Nm Pm Eb Qa	AAC(6′) APH(2″) AAD(4′)(4″)	47.8	292a, 442, 551
pSH6	Gm Tm Km	AAC(6′) APH(2″)	20.8	157, 217, 308
pSH8	Gm Tm Km Pm Eb Tra$^+$[d]	AAC(6′) APH(2″) AAD(4′)(4″)	45	217, 308
pSH9	Gm Tm Km Pm Pc Tra$^+$	AAC(6′) APH(2″) AAD(4′)(4″)	57	217, 308
	Gm Tm Km	ND	37.5	104
pUW3626	Gm Tm Km Nm Pm Eb Qa Pc Tra$^+$	ND	54.4	79, 157, 159, 292a
pTU053	Gm Tm Km Pc Em	AAC(6′) APH(2″) APH(3′)III	48	519, 520
pTU068	Gm Tm Km	AAC(6′) APH(2″)	54	519, 520
	Gm Tm Km Eb Tra$^+$	AAC(6′) APH(2″) AAD(4′)(4″)	38	11
	Gm Tm Km Eb Pc Tra$^+$	AAC(6′) APH(2″) AAD(4′)(4″)	53	11
pCRG500	Gm Tra$^+$	ND	48	159
pCRG1600	Gm Km Eb Qa Pc Tra$^+$	ND	52.9	13, 159
pSJ19	Gm Km	AAC(6′) APH(2″) APH(3′)III	44.4	169, 171
pSJ49a	Gm Km	AAC(6′) APH(2″)	42.0	169, 171
pLJM36	Gm	AAC(6′) APH(2″)	52	298
pLJM48	Gm	AAC(6′) APH(2″)	46	298
pJE2	Gm Tm Km Pc Asa Asi Cd	ND	38	339; K. Dyke, personal communication
pJE1	Gm Tm Km Pm Eb Qa Tra$^+$	ND	50	222, 339; K. Dyke, personal communication
pSK1	Gm Tm Km Ac Eb Qa Pi Dd Tp	AAC(6′) APH(2″)	28.4	295, 297; Rouch et al., submitted
pSK4	Gm Tm Km Ac Eb Qa Pi Dd Tp Pc	ND	35.1	157, 293, 295
pSK9	Gm Tm Km Ac Eb Qa	ND	25.7	293
pSK14	Gm Tm Km Ac Eb Qa	ND	24.4	293
pWG53	Gm Tm Km Ac Eb Qa Tp	ND	27	128, 504, 512
pGO1	Gm Eb Qa Tp Tra$^+$	ND	50	9

[a] See Table 1, footnote *b*, for abbreviations of antimicrobial agents.
[b] ND, Not determined.
[c] DiP$^+$, Diffusible pigment production.
[d] Tra$^+$, Self-transmissibility.

Resistance to Neomycin and Kanamycin

Sm^r plasmid pWA1 also mediates resistance to neomycin and kanamycin by encoding APH(3′)III (92). The APH(3′)III was believed to be encoded on a 1.8-kb DNA fragment which was homologous with the Nm^r Km^r plasmids pSH2 and RN1956; the latter plasmids were indistinguishable from one another, except for an additional 0.75-kb DNA segment present in RN1956 (Table 4). The gene for APH(3′)III (designated *aphA*) was cloned from pSH2 and found to confer resistance on *E. coli* (92), and the nucleotide sequence has subsequently been determined and shown to encode a protein of 30,724 molecular weight (170). An APH(3′)III of equivalent size was purified from a transductant carrying the 44.7-kb Km^r Pc^r Em^r plasmid pTU512 (520). This enzyme is also thought to be responsible for the Nm^r Km^r mediated by a group of plasmids characterized in the United States (171) and the 36-kb plasmid pTC128 detected in strains of *S. aureus* from Dublin hospitals (87).

Resistance to neomycin and kanamycin could not be associated with an extrachromosomal element in strains of *S. aureus* from Switzerland (224), France (125), and Australia (153, 154). In the case of those strains from Switzerland, a chromosomal location for *aphA* was confirmed in both the wild-type strain and Nm^r Km^r transductants (224). The cloning of a chromosomal *aphA* has recently been achieved, and subsequent hybridization analysis with wild-type and transductant strains indicated that, in all but one, the gene occupied an identical site (126a, 127). The same authors also observed the translocation of the gene from the chromosome to a β-lactamase plasmid, thereby implying that *aphA* is carried on a transposon.

A number of plasmids have been reported which mediate resistance to neomycin, kanamycin, paromomycin (Pm^r), tobramycin (Tm^r), and amikacin via a AAD(4′)(4″). These include small multicopy plasmids such as pUB110, pAP010, and pSK50 (Table 4). A restriction map of pUB110 was produced (36, 218) and the plasmid has been extensively used as a cloning vector in *B. subtilis* (36, 52, 228). The pUB110-specified AAD(4′)(4″) was characterized in this new host and shown to be a monomeric protein with a molecular

weight of 30,000 to 34,000 (303, 430); nucleotide sequencing of the structural gene (designated *aadD*) established that the AAD(4′)(4″) protein would have a molecular weight of ca. 29,000 (303). The entire sequence of pUB110 has subsequently been reported (311).

Larger, low-copy-number plasmids have also been shown to mediate aminoglycoside resistance through an AAD(4′)(4″). A series of plasmids isolated in France (pIP524, pIP630, and pIP629; Table 4), which range in size from 26.1 to 34.8 kb, encode resistance to tobramycin, streptogramins A and B, tetracycline, and cadmium (126). These plasmids belong to the same incompatibility group and share 50 to 100% base sequence homology; hence it is probable that they diverged from a common ancestor. Such plasmids are homologous with a 4.6-kb plasmid that encodes AAD(4′)(4″) activity (127), suggesting that *aadD* is present on both small and large plasmids. Large plasmids isolated from gentamicin-resistant strains of *S. aureus* from the United States also often encode an AAD(4′)(4″), in addition to an enzyme which inactivates gentamicin (11, 217, 442). The determinant for AAD(4′)(4″) has been mapped by deletion analysis on plasmids pSH8 (308) and pCRG1600 (13), as depicted in Fig. 9 by the regions marked Pm and Nm, respectively. This location corresponds to that for the genetic determinant of Tm^r Km^r Nm^r Pm^r detected on the related plasmids pSK41 and pUW3626 by DNA-DNA hybridization with pSK50, a plasmid which is seemingly identical to the *aadD*-carrying plasmid pUB110 (see Fig. 9; Gillespie and Skurray, unpublished data).

Gentamicin Resistance

Resistance to gentamicin in *S. aureus* is considered to be mediated by AAC(6′) and APH(2″) activities which result in coincident resistance to tobramycin and kanamycin (447). Attempts to separate the AAC(6′) and APH(2″) activities by biochemical means have been unsuccessful, and it has been suggested that a single bifunctional protein may catalyze both reactions (281, 300, 520).

Studies on Gm^r Tm^r Km^r *S. aureus* have demonstrated that, in the majority of strains, the genetic determinant for AAC(6′) and APH(2″) (designated *aacA-aphD*) is plasmid-borne. The first reported Gm^r Tm^r Km^r plasmid was isolated in France (470), and since that time such plasmids have been detected in strains from the United States (527, 551), the United Kingdom (104), Japan (519), Australia (294, 512), and East and West Germany (339; W. Witte, personal communication).

Many of the Gm^r Tm^r Km^r plasmids isolated in the United States have been extensively characterized and shown to possess a high degree of structural relatedness. In a comprehensive analysis of 41 representative Gm^r *S. aureus* and coagulase-negative staphylococcal strains, Jaffe and colleagues (217) demonstrated a family of structurally similar Gm^r plasmids which could be divided into five classes on the basis of phenotype and endonuclease digestion pattern. These plasmids included the 20.8-kb Gm^r plasmid pSH6, the 45-kb Gm^r Pm^r plasmid pSH8, and the 57-kb Gm^r Pm^r Pc^r plasmid pSH9, the latter two of which have been shown to be self-transmissible (Table 4) (308). Substantial identity was also observed between the restriction endonuclease maps of staphylococcal conjugal Gm^r plasmids when Goering et al. (160) compared plasmids isolated from different geographical regions of the United States. Among the plasmids studied were the *S. aureus* plasmids pCRG1600 (159), pUW3626 (79), and pSH8 (308) and the *S. epidermidis* plasmids pAM899-1 (141) and pG02 (9). Plasmids pCRG1600 and pUW3626 were found to be virtually identical and only differed significantly from pSH8 with respect to a 6.6-kb insertion in *Eco*RI fragment B, now associated with the Pc^r transposon, Tn*4201* (Weber and Goering, 25th ICAAC), which was absent in the latter. The two *S. epidermidis* plasmids were apparently indistinguishable and exhibited much identity with pSH8.

Three of the Gm^r plasmids isolated in the United States have been mapped in greater detail in our laboratory (292a). Plasmids pUW3626 and pSK41 (the Gm^r plasmid detected in strain Spratlin [551]) proved to be identical except for the presence of Tn*4201* in the former, and both were obviously homologous with pSH6 (Fig. 9). Comparison of these maps with the reported maps of pSH8 (308), pG01 (9), and pCRG1600 (13) exemplifies the relatedness of all of these Gm^r plasmids (Fig. 9) and confirms the notion of a clonal origin for the Gm^r plasmids isolated from staphylococcal strains in the United States (79).

On the basis of restriction mapping, plasmids such as pSK41 and pSH8 also clearly resemble pSJ19 (Fig. 9), the prototype of a family of Gm^r Km^r Tm^r plasmids characterized by Gray and others (169, 171). Members of the pSJ19 plasmid family were shown to be compatible (since they were natural coresidents) with alpha family β-lactamase plasmids homologous with pI524, thereby implying that they do not belong to the IncI incompatibility group. In addition to encoding penicillin and heavy-metal resistances, the coresident alpha family plasmids, of which pSJ24 is an example (Table 2), carried the genes for AAC(6′) and APH(2″), or APH(3′)III, or all three, suggesting that exchange of genetic material between the two different replicons had occurred (169, 171).

In Australian strains of *S. aureus*, Gm^r Tm^r Km^r is mediated by a distinct family of plasmids represented by the 28.4-kb prototype pSK1 (Table 4) (293, 295). Such plasmids are structurally unrelated to their U.S. counterparts (Fig. 9), and unlike the latter, are nonconjugal and belong to the IncI incompatibility group. In plasmids such as pSK1 and pSK4, Gm^r Tm^r Km^r was shown to be correlated with the presence of a 4.7-kb segment of DNA which was absent in the homologous Gm^s Tm^s Km^s plasmid pSK7 (Fig. 9). An apparently identical 4.7-kb DNA segment was also associated with Gm^r Tm^r Km^r in pSK23 (Table 2), a heavy-metal resistance plasmid similar to pSJ24 (Fig. 2). Plasmids analogous to the pSK1 family and to pSK23, among which are pWG53 (Table 4) and pWG50 (Table 2), respectively, have been reported by Grubb and co-workers (504, 512).

Digestion of Gm^r Tm^r Km^r plasmids from various sources with the restriction endonuclease *Hin*dIII has revealed that they each possess a characteristic 2.3- to 2.5-kb *Hin*dIII fragment (217, 295, 298, 339). A fragment of this size was associated with Gm^r Tm^r Km^r in physical maps of the *S. aureus* plasmid pSH8 (308) and the *S. epidermidis* plasmid pG02 (10), and it has been reported that the gene for AAC(6′) and APH(2″) was introduced into *E. coli* and *B. subtilis* by the cloning of a 2.3-kb *Hin*dIII fragment from a pSJ19-like plasmid (171). In addition, studies in our laboratory have demonstrated that a 2.5-kb *Hin*dIII fragment associated with Gm^r Tm^r Km^r in pSK1 (296) can express AAC(6′) and APH(2″) activities from the natural promoter when cloned in *E. coli* (D. Rouch, M. Byrne, Y.-C. Kong, and R. Skurray, submitted for publication). This fragment has subseqently been shown to be homologous with fragments of equivalent size in *Hin*dIII digests of the U.S. plasmids pSH6, pSK41, and pUW3626 (Fig. 9), thereby implying that the determinants of Gm^r Tm^r Km^r from these diverse replicons are

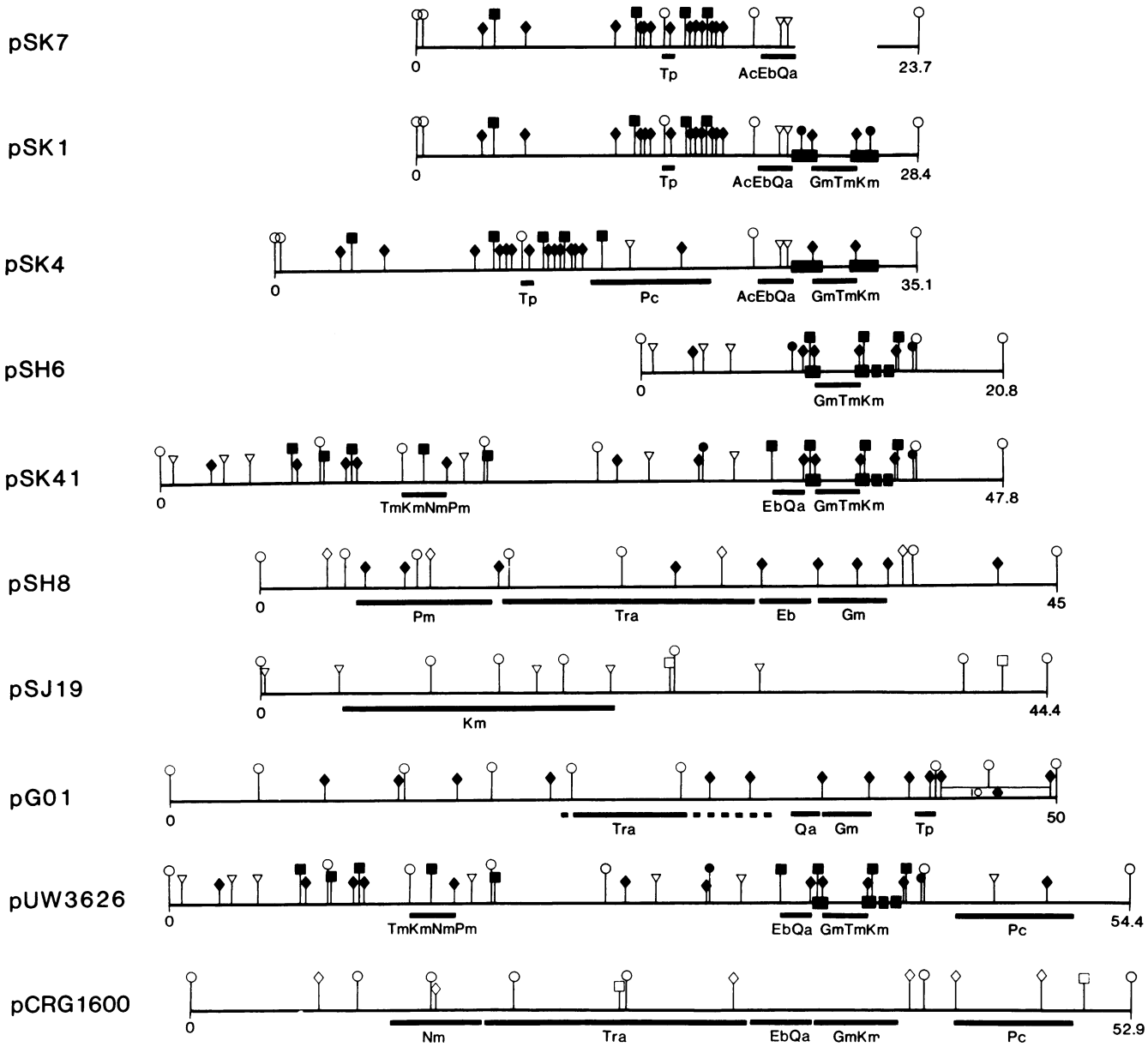

FIG. 9. Linear restriction maps of plasmids pSK7, pSK1, pSK4, pSH6, pSK41, pSH8, pSJ19, pGO1, pUW3626, and pCRG1600 (9, 13, 157, 169, 292a, 297, 308). All maps are aligned relative to the 2.5-kb *Hin*dIII fragment within the Gm^r Tm^r Km^r region of pSK1; coordinates are in kilobases. Restriction endonuclease sites are indicated by: ■ (*Bgl*II), ○ (*Eco*RI), ◆ (*Hin*dIII), □ (*Kpn*I), ▽ (*Pvu*II), and ◇ (*Xba*I). Ten unordered *Hin*dIII sites (10 ◆) are indicated on pG01. Gm,Tm, Km, Ac, Eb, Qa, Tp, Nm, Pm, and Pc designate the plasmid DNA segments (indicated by solid lines below the map) that mediate resistance to gentamicin, tobramycin, kanamycin, acriflavine, ethidium bromide, quaternary ammonium compounds, trimethoprim, neomycin, paromomycin, and penicillin, respectively. Tra indicates the DNA segment that encodes plasmid self-transmissibility. Inverted repeats bounding or adjacent to the Gm^r region are represented by the closed boxes.

identical (292a). Such evidence further raises the possibility that *aacA-aphD* is located within a transposon.

Gentamicin resistance in a number of clinical isolates of European origin could not be linked to a plasmid locus (87, 108, 125, 339, 390, 414, 549). In one instance, the genetic determinant for production of AAC(6′) and APH(2″) was presumed to be located on the chromosome in the parental strain and a subsequent transductant (224). Evidence has also been presented which links Gm^r with a prophage genome and suggests that insertion of the resistance determinant into the chromosome following transduction may occur at different loci (266). More recently, strains from France have been found to possess a chromosomally encoded *aacA-aphD* determinant, and this has been cloned and shown to express in *E. coli* and *B. subtilis* (126a, 127).

Chromosomal Gm^r Tm^r Km^r is also exhibited by some Australian isolates of *S. aureus* (153, 295, 512), and similarities between the aminoglycoside MICs for these strains and those bearing plasmids such as pSK1 and pWG53 led to speculation that the Gm^r Tm^r Km^r determinants are the same. Probing of restricted DNA from a number of clinical isolates which demonstrated chromosomal resistance, with a

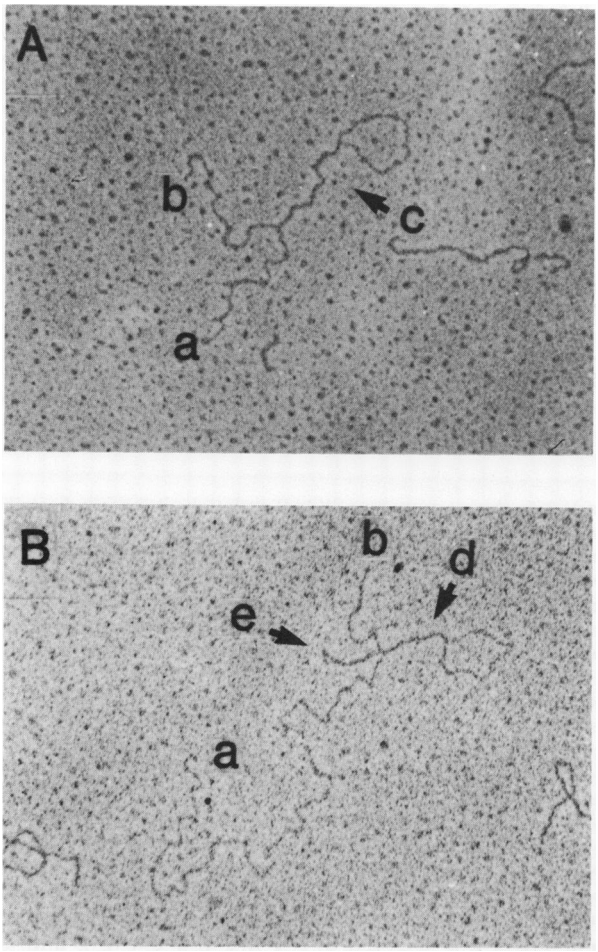

FIG. 10. Electron micrographs of *Eco*RI-cleaved, self-annealed pSK1 (A) and pSH6 (B) plasmid DNA. The ends of each molecule are denoted by a and b. The arrow marked c indicates the 4.7-kb stem and loop structure of Tn*4001*; the arrow marked d indicates a structure with a similar sized loop to Tn*4001*, but with a shorter stem. These stem and loop structures have been located on the respective plasmid restriction maps and correspond to the position of the common 2.5-kb *Hin*dIII fragment associated with Gm^r Tm^r Km^r (Fig. 9). The role of the additional stem and loop structure (e) detected on U.S. plasmids pSH6, pSK41, and pUW3626 is unknown.

Gm^r Tm^r Km^r-specific fragment derived from pSK1, has confirmed that the chromosomal and plasmid determinants share homology. Moreover, the determinant was shown to be located at distinct sites on the *S. aureus* chromosome in different strains (M. Gillespie, B. Lyon, L. Messerotti, and R. Skurray, J. Med. Microbiol., in press).

By a series of genetic tests involving transposition to at least four sites in the penicillin and heavy-metal resistance plasmid pII147, together with *rec*-independent transposition to the chromosome, we have established that Gm^r Tm^r Km^r mediated by pSK1 is encoded on a transposable element designated Tn*4001* (296). Physical and genetic mapping of chromosomal Tn*4001* insertions produced in the laboratory has demonstrated that this transposon displays a low specificity of insertion in the staphylococcal chromosome (Fig. 1; G. Mahairas, B. Lyon, R. Skurray, and P. Pattee, submitted for publication), a finding consistent with the diversity of sites assumed to be occupied by the element in clinical isolates. The low specificity of Tn*4001* insertion makes this element an excellent candidate for transposon mutagenesis studies of *S. aureus* plasmid and chromosomal genes. Tn*4001* has yet to be compared with the Gm^r Tm^r Km^r transposon Tn*3851* described by Grubb and colleagues (505), but from the reported characteristics the two appear to be very similar.

Tn*4001* measures 4.7 kb in size and consists of a unique 2.0-kb region which encodes Gm^r Tm^r Km^r, flanked on either side by inverted repeat sequences of 1.35 kb. These inverted repeats cause the formation of a characteristic stem and loop structure when self-annealed molecules of the transposon are viewed by electron microscopy (Fig. 10A). Symmetrical *Hin*dIII sites located within the inverted repeats of Tn*4001* were found to result in the production of the 2.5-kb *Hin*dIII fragment associated with Gm^r Tm^r Km^r in the pSK1-like plasmids (Fig. 9). Although Gm^r Tm^r Km^r plasmids isolated in the United States also possessed this 2.5-kb *Hin*dIII fragment (Fig. 9), and electron microscopic analyses of plasmids such as pSH6 (Fig. 10B), pSK41, and pUW3626 revealed the presence of inverted repeats flanking a Gm^r Tm^r Km^r region of similar size to that seen in Tn*4001*, such repeats were shown to be substantially shorter than those present in Tn*4001*. In fact, the inverted repeats in the U.S. plasmids only measured 0.7 kb, giving a total stem and loop size of approximately 3.6 kb (292a). Hybridization analysis has demonstrated homology between the inverted repeats of Tn*4001* and the transposon-like structures of the U.S. plasmids; however, the presence of *Bgl*II sites in the latter, which are absent in the former (Fig. 9), implies that there are sequence differences between the two. All attempts to achieve the transposition of the U.S. Gm^r Tm^r Km^r determinant, both in our hands and in others (11), have been unsuccessful, and the possibility exists that the shorter inverted repeats do not encode the necessary functions for transposition.

Evidence has accrued which implies that at least one of the inverted repeats of Tn*4001* can act independently of the transposon in the manner of an insertion sequence (B. Lyon, M. Gillespie, and R. Skurray, submitted for publication). The inverted repeat of Tn*4001* has accordingly been designated IS*256*. The molecular structure of Tn*4001* thus resembles that of composite or class I transposons such as Tn*5* and Tn*10*, which also possess an antibiotic resistance determinant(s) flanked by inverted insertion sequences (238).

An apparently contiguous, direct tandem duplication of IS*256* on pSK1 (Fig. 11) was detected by DNA-DNA hybridization and the visualization of self-annealed plasmid molecules. Such duplication was shown to result in an increase in the level of expression of Gm^r Tm^r Km^r, a finding which suggests that IS*256* contains promoter sequences capable of acting in tandem to augment transcription of adjacent genes. A similar phenomenon was observed among strains of *S. aureus* isolated in the United Kingdom, where plasmids possessing an additional 0.75 kb of DNA exhibited a fourfold increase in gentamicin resistance (503). The independent presence of IS*256* at multiple sites on the chromosomes of clinical isolates of multiresistant *S. aureus* has also been detected by DNA-DNA hybridization, thereby providing the first indication that insertion sequences may be actively involved in molecular rearrangements of the staphylococcal genome.

The cloning and analysis in *E. coli* of the Gm^r Tm^r Km^r-encoding region of Tn*4001* and the subsequent establishment of the DNA sequence of this region have provided additional data on the genetic and molecular basis of this

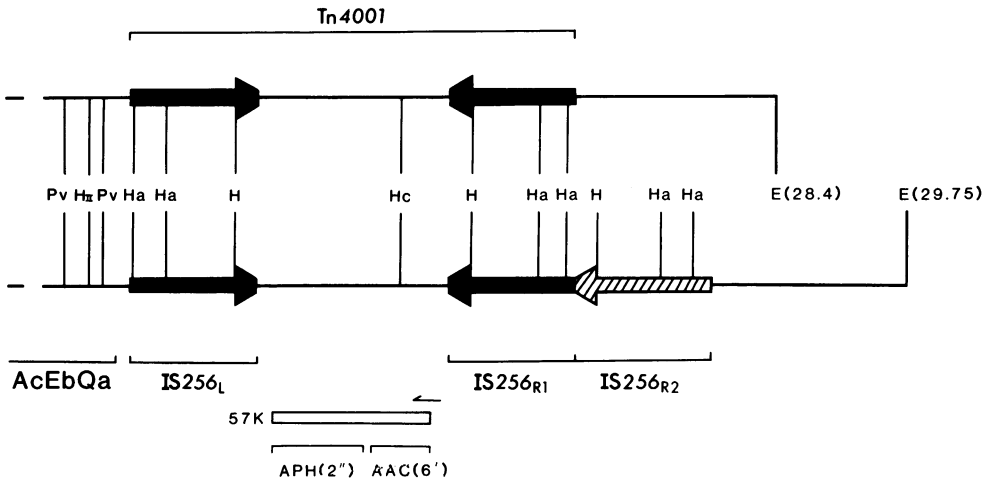

FIG. 11. Restriction map of the contiguous, direct tandem duplication of IS*256* which occurs on pSK1. Restriction sites are indicated by E (*Eco*RI), H (*Hin*dIII), H_{II} (*Hpa*II), Ha (*Hae*III), Hc (*Hin*cII), and Pv (*Pvu*II); a complete map of the 28.4-kb plasmid pSK1 is shown in Fig. 9. AcEbQa indicates part of the region associated with resistance to acriflavine, ethidium bromide, and quaternary ammonium compounds. The 1.35-kb inverted repeats of Tn*4001* ($IS256_L$ and $IS256_{R1}$) are represented by the bold arrow, and the duplicated insertion sequence ($IS256_{R2}$) is represented by the hatched arrow; the directions of transcription from putative promoter sequences within the IS*256* elements are indicated by the arrowheads. The small arrow indicates the direction of transcription of the *aacA-aphD* gene of Tn*4001*, while the open box represents the 57,000 (57K)-molecular-weight translational product (Rouch et al., submitted). The domains for the AAC(6′) and APH(2″) enzyme activities of this bifunctional protein are indicated.

resistance (Rouch et al., submitted). It has been proposed that Gm^r Tm^r Km^r in *S. aureus* is mediated by a bifunctional protein of molecular weight 54,000 to 56,000 which exhibits AAC(6′) and APH(2″) activities (300, 520). Polypeptide analysis of the cloned 2.5-kb *Hin*dIII fragment of Tn*4001* has indicated the involvement of a single 59,000-molecular-weight protein in resistance to the three aminoglycosides. Moreover, transposon insertion mutagenesis has demonstrated the direction of transcription of the *aacA-aphD* gene on this cloned fragment and confirmed the suggestion (300, 520) that the protein it encodes possesses two domains, one of which produces the Km^r Tm^r phenotype via the AAC(6′) activity and a second which mediates Gm^r via the APH(2″) activity (Fig. 11). DNA sequence analysis of Tn*4001* indicated that the AAC(6′)-APH(2″) polypeptide is composed of 479 amino acids with a predicted molecular weight of 57,000 and that sequences within the AAC(6′) domain are essential for the correct folding of the putative APH(2″) active site (Fig. 11; Rouch et al., submitted).

RESISTANCE TO ACRIFLAVINE, ETHIDIUM BROMIDE, AND QUATERNARY AMMONIUM COMPOUNDS

Staphylococcal resistance to the intercalating agents acriflavine (Ac^r) and ethidium bromide (Eb^r) was first associated with the presence of β-lactamase plasmids >15 years ago (132, 220), and in recent times resistance to the latter has often been observed coincidently with gentamicin resistance (11, 13, 87, 296, 308, 507). In some instances, concomitant resistance to a number of related compounds, among them quaternary ammoniums (Qa^r), such as cetyltrimethylammonium bromide (cetrimide) and benzalkonium chloride, propamidine isethionate (Pi^r), and diamidinodiphenylamine dihydrochloride (Dd^r), was also reported (13, 128, 296), as was resistance to chlorhexidine (53, 87).

Earlier studies indicated that the mechanism of Eb^r involved diminished uptake of the agent (220, 308), and subsequent experiments have established that an efflux system, similar to that responsible for resistance to cadmium, tetracycline, and arsenate, achieves this effect (222; M. Midgley and G. Jones, personal communication). Such a mechanism implies that, in the sensitive organism, the target of ethidium bromide and related compounds must be located intracellularly. It has been suggested that resistance to these compounds was selected for by the frequent use, until the mid-1970s, of agents including acriflavine and cetrimide as antiseptics and disinfectants (142, 491). The demonstrated synergism between cetrimide and selected antibiotics against *Pseudomonas aeruginosa* (122) may provide a further explanation for the prevalence of Qa^r in hospital staphylococci.

Genetics of Quaternary Ammonium Resistance

Genetic and molecular analysis of *S. aureus* isolates exhibiting resistance to agents such as ethidium bromide and quaternary ammonium compounds has identified at least three distinct resistance determinants (128, 129, 157; Gillespie and Skurray, unpublished data). The first of these, which we have designated *qacA*, mediates Ac^r Eb^r Qa^r Pi^r Dd^r and is located on all of the pSK1 family plasmids. This determinant is presumed to be equivalent to that responsible for the type I nucleic acid binding resistance reported for pWG53 (Table 4) (130). The *qacA* determinant derived from pSK1 has been extensively studied in our laboratory. A 3.4-kb segment of pSK1 DNA which encodes Ac^r Eb^r Qa^r Pi^r Dd^r was shown to express this phenotype when cloned in an *E. coli* vector/host system (491). Transposon insertion mutagenesis of this cloned region has indicated that a single determinant, occupying at least 1.25 kb, encodes resistance to these agents (Fig. 12). Restriction mapping and DNA-DNA hybridization have revealed that the *qacA* determinant is also present on a number of β-lactamase/heavy-metal resistance plasmids including pSK57 (Table 2; Fig. 2 and 12), an alpha/gamma recombinant plasmid which shares >60% identity with pI524 (155).

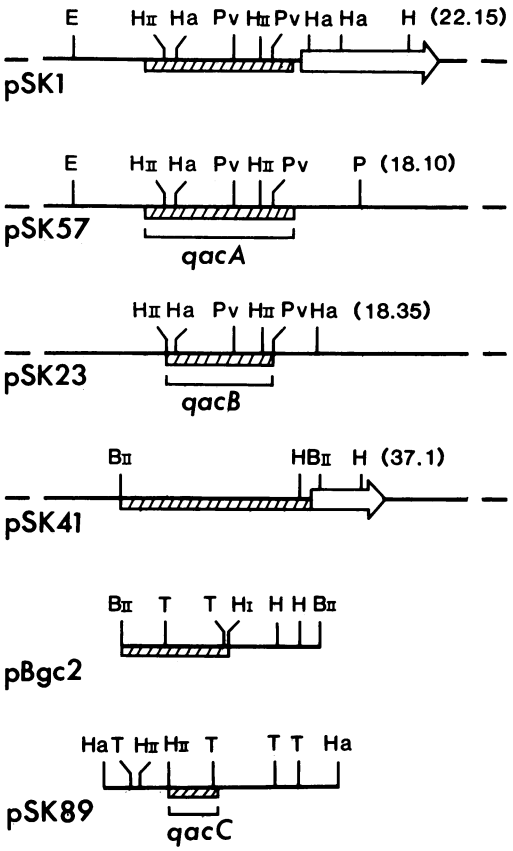

FIG. 12. Comparative restriction maps of quaternary ammonium resistance regions of six different Qa^r plasmids. Map coordinates are expressed in kilobases and correspond with those used in the complete maps (Fig. 2 and 9). Restriction sites are indicated by B_{II} (*Bgl*II), E (*Eco*RI), H (*Hin*dIII), H_I (*Hpa*I), H_{II} (*Hpa*II), Ha (*Hae*III), P (*Pst*I), Pv (*Pvu*II), and T (*Taq*I). In each case, the hatched region represents the extent of the Qa^r region as determined by mapping, subcloning, hybridization, or transposon mutagenesis. On the basis of phenotype and DNA homology, *qacA* is present on pSK1 and pSK57; *qacB*, on pSK23; and *qacC*, on pSK41, pBgc2, and pSK89 (see text). Open arrows indicate the locations of inverted repeat sequences associated with the Gm^r determinants of pSK1 and pSK41 (see Fig. 9).

Similar techniques have detected a region with DNA sequences homologous to those of *qacA* on the alpha/gamma recombinant plasmid pSK23 (Fig. 2 and 12) (157). However, this plasmid only mediated Ac^r Eb^r Qa^r and therefore is thought to possess a slightly different resistance determinant defined as *qacB*. This determinant is probably also present on the penicillin/heavy-metal resistance plasmid pWG50 (Table 2) (128).

The third determinant, designated *qacC*, does not demonstrate homology with the first two and mediates Qa^r and low-level Eb^r. It has been detected on conjugative Gm^r Tm^r Km^r plasmids isolated in the United States (e.g., pSK41 and pUW3626) and is also carried by small plasmids from Australian strains, such as pSK89 (Table 3). The resistance phenotype of *qacC* is equivalent to the reported type II nucleic acid binding resistance phenotype (130). Four distinguishable type II nucleic acid binding resistance plasmids which range in size from 2.24 to 2.88 kb have also been identified in strains of *S. aureus* from Australia, Italy, and the United States (130). pSK89 (Fig. 12) is equivalent to one of these plasmids, pWG32, on the basis of restriction mapping (Gillespie and Skurray, unpublished data), thus confirming the identity of the type II nucleic acid binding and *qacC* resistance determinants.

Hybridization with labeled pSK89 DNA localized the *qacC* determinant of the aminoglycoside resistance plasmids pSK41 and pUW3626 to a 2.0-kb *Bgl*II fragment which maps adjacent to the Gm^r Tm^r Km^r determinant (Fig. 9; Gillespie and Skurray, unpublished data). This location corresponded with the positions of the Eb^r and Qa^r determinants of pSH8 and pCRG1600 which were previously mapped by deletion analysis (13, 308); hence, these plasmids are also likely to encode *qacC*. Significantly, the Eb^r Qa^r determinant of the Gm^r plasmid pJE1, which was isolated in West Germany (339) and appears physically related to conjugative Gm^r plasmids from the United States (K. Dyke, personal communication), has been cloned in *E. coli* on a 2.0-kb *Bgl*II fragment (222). A 1.1-kb *Bgl*II-*Hpa*I subcloned fragment from this *E. coli* clone, pBgc2 (Fig. 12), was shown to express full resistance, thereby suggesting that a single polypeptide of not more than 350 amino acid residues confers resistance to both ethidium bromide and quaternary ammonium compounds. It is tempting to speculate that this Eb^r Qa^r determinant is equivalent or very closely related to *qacC*, despite some minor differences in their restiction sites (Fig. 12).

RESISTANCE TO THE SULFONAMIDES AND TRIMETHOPRIM

Both the sulfonamides and trimethoprim achieve their antibacterial effect by interfering with the biosynthesis of tetrahydrofolic acid, derivatives of which are essential for the synthesis of several amino acids and nucleotides (142, 464). An early step in the synthesis of tetrahydrofolic acid is the condensation of dihydropteridine with *p*-aminobenzoic acid by the enzyme dihydropteroate synthase (DHPS), which results in the generation of dihydropteroic acid. The sulfonamides are analogs of *p*-aminobenzoic acid and act to competitively inhibit DHPS and deplete the supply of dihydropteridine by the formation of an inactive sulfonamide analog of dihydropteroic acid. The final step of the tetrahydrofolic acid pathway involves the reduction of dihydrofolic acid to the activated tetrahydrofolate form by the enzyme dihydrofolate reductase (DHFR). Trimethoprim is an analog of dihydrofolic acid and causes competitive inhibition of DHFR due to a 1,000-fold-higher affinity for the enzyme than the natural substrate (142).

Sulfonamide Resistance

At least two different mechanisms of bacterial resistance to the sulfonamides (Su^r) have been reported; however, it is not known whether both are operative among the staphylococci. Strains of *S. aureus* in which Su^r was associated with an increased production of *p*-aminobenzoic acid were observed soon after the introduction of these agents (276). Resistance of this type is probably due to a chromosomal mutation (*sulA*). Plasmid-encoded Su^r is common in gram-negative bacteria and is mediated by DHPS enzymes with much reduced affinity for the drug (488). Only one Su^r plasmid, the 2.85-kb Su^r Em^r plasmid pWA2, has been described in *S. aureus* (92), and no suggestion of a possible mechanism of resistance was made.

Trimethoprim Resistance

Resistance to trimethoprim (Tp^r) in gram-negative organisms can result from the overproduction of the normal

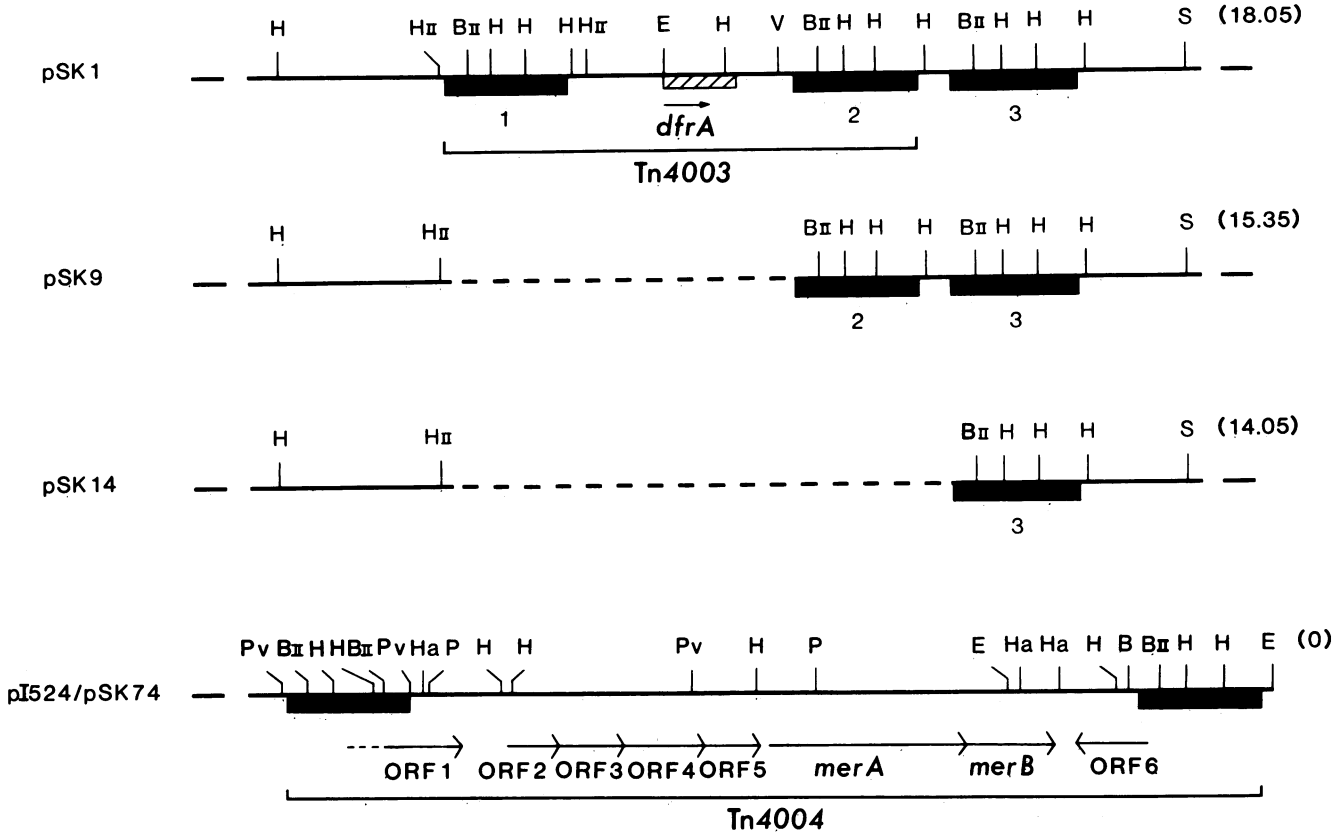

FIG. 13. Physical map of the Tp^r region of pSK1 aligned with maps of the corresponding regions of the naturally occurring Tp^s variant plasmids pSK9 and pSK14. Coordinates are in kilobases; restriction endonuclease sites are represented by B (*Bam*HI), B_{II} (*Bgl*II), E (*Eco*RI), H (*Hin*dIII), H_{II} (*Hpa*II), Ha (*Hae*III), P (*Pst*I), Pv (*Pvu*II), S (*Sal*I), and V (*Eco*RV). The extent of the structural gene for Tp^r (*dfrA*) is indicated by the hatched region, while the arrow shows the direction of transcription. The directly repeated IS*257* elements identified on each plasmid are represented by the solid boxes; the structure of the putative transposon Tn*4003* is labeled. Plasmids pSK9 and pSK14 only differ from pSK1 through the absence of the regions indicated by the dotted lines, which presumably represent deletions of the latter caused by reciprocal recombination between IS*257* elements on either side of the Tp^r region. Also shown is a map of the *mer* region of pI524/pSK74 (Tn*4004*) indicating the proposed locations of the directly repeated IS*257*-like element (Gillespie et al., submitted). The extent and directions of transcription of the *merA* and *merB* genes and six ORFs (ORF1 to 6) detected by sequencing of the *mer* region of pI258 (Laddaga et al., submitted) are illustrated below.

chromosomal DHFR (61), but is usually mediated by plasmids which encode drug-resistant DHFR enzymes (464). The former mechanism is characteristically associated with a low level of resistance (MIC, 10 to 500 μg/ml), whereas the latter produces a high level of resistance (MIC, ≥1,000 μg/ml) (258). Reports of Tp^r in *S. aureus* have indicated that this phenotype can be of the "intrinsic" or chromosomally mediated type (284, 291, 295, 504) or is encoded on large plasmids (9, 87, 297, 504). As in the gram-negative bacteria, chromosomal and plasmid-mediated Tp^r in *S. aureus* can be distinguished on the basis of MICs, and although the mechanism of chromosomal Tp^r has not been determined, plasmid-mediated resistance in strains isolated in Australia and the United States is due to a unique DHFR (type S1) with markedly decreased affinity for trimethoprim (H.-K. Young, R. A. Skurray, and S. G. B. Amyes, submitted for publication; G. Archer, personal communication).

Strains of multiresistant *S. aureus* isolated in Australia are noteworthy in that individual organisms may exhibit both chromosomal and plasmid-mediated Tp^r (295, 297, 504). The chromosomal resistance determinant, tentatively designated *dfrB*, exerts low-level Tp^r (MIC, <100 μg/ml), possibly due to DHFR overproduction. High-level Tp^r (MIC, >800 μg/ml) is mediated by some members of the pSK1 plasmid family, including the prototype and pSK4 (Table 4). By comparing the restriction map of pSK1 with that of the naturally occurring Tp^s variant pSK9, it was possible to localize the Tp^r determinant (designated *dfrA*) to a 2.75-kb region of pSK1 (Fig. 13). Relevant fragments of pSK1 have been cloned and shown to express the Tp^r phenotype in an *E. coli* host/vector system. Subsequent transposon mutagenesis of one clone has limited the extent of *dfrA* to between 0.55 and 0.75 kb and determined the direction of transcription (Fig. 13) (297). Type S1 DHFR from pSK1 has a molecular weight of 19,700 and, on the basis of the minimum size of *dfrA* (sufficient to encode a polypeptide of ca. 20,000 molecular weight), is presumed to consist of a single subunit of the Tp^r polypeptide (Young et al., submitted). High-level plasmid-mediated Tp^r was also detected in 10% of *S. aureus* isolates from hospitals in the United States and the Tp^r determinant from one such plasmid, pG01 (Fig. 9), has similarly been cloned and shown to express resistance in *E. coli* (9). Restriction data suggest that the Tp^r determinant from pG01 closely resembles *dfrA*, and indeed strong hybridization signals were detected between the Tp^r region of pG01 and the pSK1-like plasmid pWG53 (9).

Restriction mapping of the Tp^r region of pSK1 has revealed the presence of three directly repeated sequences of

ca. 920 bp which flank the Tp^r gene (Fig. 13). Surprisingly, these direct repeats have been shown, by restriction mapping and DNA-DNA hybridization, to be indistinguishable from directly repeated elements which flank the *mer* operon in β-lactamase plasmids, such as pI524 and pSK74 (Gillespie et al., submitted), and which are also detected adjacent to the chromosomal *mec* and *tet* determinants (28; Matthews et al., submitted; Gillespie et al., submitted) (see relevant sections). These direct repeats have accordingly been designated IS*257*, and their arrangement in pSK1 suggests that the Tp^r gene may form part of a composite transposon, tentatively named Tn*4003*, which possesses copies of IS*257* in the form of direct repeats at either end. The presence of the third copy of IS*257* could be explained by an independent insertion or duplication of one of these IS elements, a situation not unlike the duplication of IS*256* in Tn*4001*. Independent insertion of IS*257* sequences also appears to have occurred at multiple sites on transmissible Gm^r plasmids, such as pSH6, pSK41, and pUW3626, detected in U.S. isolates (Gillespie and Skurray, unpublished data). Although the physical data allude to the existence of a transposon which mediates Tp^r, no genetic proof for the transposition of such an element has been obtained.

RESISTANCE TO FUSIDIC ACID, RIFAMPIN, AND VANCOMYCIN

Other antibiotics which have been used for the clinical treatment of *S. aureus* infections include fusidic acid, rifampin, and vancomycin. To date, staphylococcal resistance to only the first two agents has been encountered to any great extent.

Fusidic Acid Resistance

Fusidic acid inhibits protein synthesis at the level of the 50S ribosomal subunit by interfering with the ribosome-associated activity of elongation factor G, the translocation protein (142). Fusidic acid resistance (Fa^r) in *S. aureus* can be due to a chromosomal mutation (*fusA*; Fig. 1) which has been postulated to lower the affinity of the G factor for the antibiotic or to a plasmid-borne determinant (*fusB*) which possibly encodes for decreased permeability towards fusidic acid (71). Linkage of Fa^r with β-lactamase and heavy-metal ion resistance plasmids, such as pUB101 (262, 265, 270) (Table 2) and the aminoglycoside resistance plasmid pWA1 (92) (Table 4), has been observed.

Rifampin Resistance

Rifampin inhibits bacterial RNA synthesis by binding to the β subunit of DNA-dependent RNA polymerase and preventing initiation of transcription (147); the mechanism of rifampin resistance (Rf^r) in *S. aureus* has not been reported but presumably results from an altered RNA polymerase (β subunit) with reduced affinity for the antibiotic (325, 399). When used as the sole agent, resistance to rifampin develops rapidly in vivo (259, 299, 448), and spontaneous Rf^r mutants of *S. aureus* have been shown to possess alterations to chromosomal rather than plasmid DNA (325). The decreased virulence of Rf^r staphylococci (323) suggests that resistance derives from a chromosomal mutation (presumably *rif*, mapping near *fusA* [Fig. 1]) which would, in the absence of selection pressure, severely disadvantage the organism. Lacey (259), on the other hand, has drawn attention to the normal growth characteristics and potential pathogenicity of Rf^r strains.

Vancomycin Resistance

Vancomycin acts primarily as an inhibitor of cell wall synthesis probably by binding to the acetyl-D-alanyl-D-alanine terminus of peptidoglycan subunits and sterically hindering enzymatic reactions which occur at the cytoplasmic membrane and are necessary for the growth of peptidoglycan chains (24, 400). Since this mechanism of action is distinct from that of β-lactam antibiotics, vancomycin has been used for the treatment of severe infections caused by methicillin- and β-lactam-resistant staphylococci (65, 236, 469, 528). Modern preparations of vancomycin are considered less toxic than earlier, unpurified formulations (65, 135, 236; Editorial, Lancet i:677–678, 1985).

There are only a few reports of vancomycin resistance (Vm^r) among *S. aureus* isolates (see reference 529 for a review of published susceptibility data). As pointed out by Reynolds (400), mutations which would lead to any change in the acetyl-D-alanyl-D-alanine target, and so reduce complexing with the antibiotic, would also affect peptidoglycan synthesis and hence be lethal for the cell. In *S. aureus*, Vm^r could potentially arise through altered permeability and failure of the antibiotic to reach the target, but this would require a major change to the outer surface of the cell since the exclusion limit of gram-positive organisms is greatly in excess of the size of vancomycin; gram-negative bacteria are intrinsically Vm^r due to the impermeability of their outer membrane (370, 400). In some instances, unsuccessful vancomycin therapy has been associated with *S. aureus* strains which displayed tolerance to the lethal action of vancomycin (136, 162).

ORIGINS AND EVOLUTION OF *S. AUREUS* RESISTANCE DETERMINANTS

The genetic determinants of resistance to many antimicrobial agents are believed to have evolved prior to the era of antibiotic chemotherapy in organisms other than those in which they are now commonly detected (for reviews, see references 142 and 239). A number of the resistance mechanisms exhibited by antibiotic-resistant pathogens are similar to those used by antibiotic-synthesizing members of the genus *Streptomyces* to protect their own cellular functions (96). For example, the 23S rRNA of streptomycetes which synthesize erythromycin is methylated at the adenine residue, as in MLS^r staphylococci and streptococci (146, 168), and the streptomycetes that produce aminoglycoside antibiotics, such as streptomycin, kanamycin, and neomycin, are known to synthesize phosphotransferases and acetyltransferases with in vitro activity against these drugs (200, 495). Many soil bacteria which compete with antibiotic-producing organisms have also been found to maintain plasmids encoding antibiotic resistance, presumably to help them survive in the environment (33, 37, 198, 202, 387). The transmission of antibiotic resistance genes from the streptomycetes and soil bacteria to clinically important species may have occurred through the exchange of plasmids or transposable elements or both in relatively more recent times (142, 354).

Intergeneric Transfer of Antibiotic Resistance Genes

Some evidence for the exchange of resistance determinants and replicons between soil bacteria and staphylococci has been reported. Comparison of the amino acid sequences of the β-lactamases produced by *B. licheniformis* and *S. aureus* has indicated that both most likely diverged from a

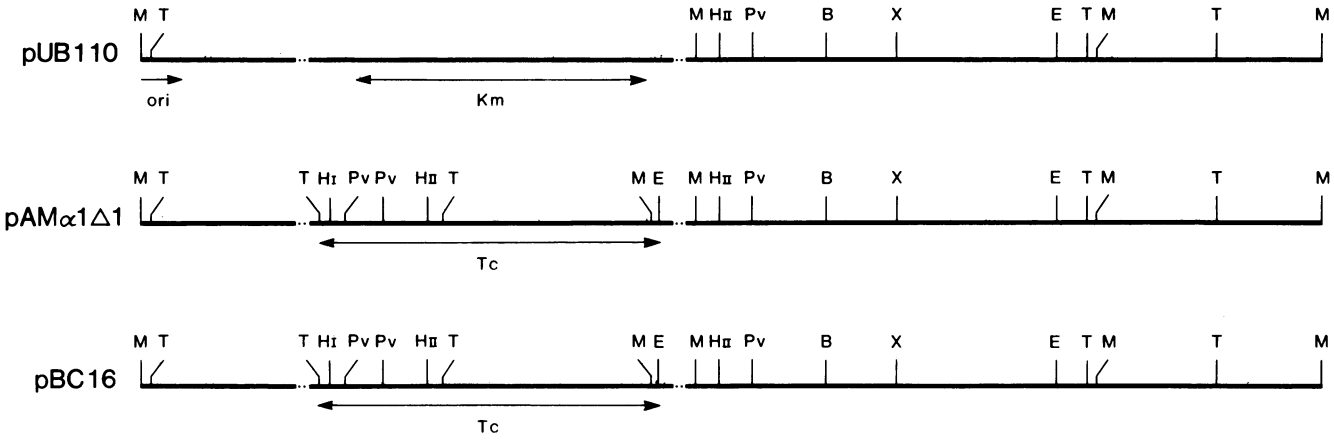

FIG. 14. Genetic and restriction maps of pUB110, pAMα1Δ1, and pBC16 aligned to emphasize identity (adapted from reference 379). Restriction sites are indicated by B (*Bam*HI), E (*Eco*RI), H_I (*Hpa*I), H_{II} (*Hpa*II), M (*Mbo*I), Pv (*Pvu*II), T (*Taq*I), and X (*Xba*I). Km and Tc designate the plasmid DNA segments that mediate resistance to kanamycin and tetracycline, respectively; *ori* refers to the origin of pUB110 DNA replication.

single ancestral gene, and a close resemblance has also been noted with the *B. cereus* β-lactamase (3). Several plasmids of staphylococcal origin, including the Tc^r plasmid pT127 and the Cm^r plasmids pC194, pC221, pC223, and pUB112 (Table 3), have been transformed into *B. subtilis* and shown to replicate in, and confer antibiotic resistance upon, the new host (120). In addition, the 4.5-kb Nm^r Km^r *S. aureus* plasmid pUB110 (72, 178) and the 4.6-kb Tc^r *B. cereus* plasmid pBC16 (33) were found to be replication incompatible in *B. subtilis* and completely homologous, with the exception of the regions encoding the different resistance phenotypes (Fig. 14) (387). Plasmid pBC16 has subsequently been shown to be virtually identical to pAMα1Δ1 (Fig. 14), a Tc^r dissociable component of the *Streptococcus faecalis* plasmid pAMα1, which is incapable of autonomous replication in the streptococci (379). Although pBC16 and the *S. aureus* Tc^r plasmid pT181 appeared to be unrelated (387), comparison of nucleotide sequences has indicated that the Tc^r determinant of another *Bacillus* plasmid, pTHT15, could produce a polypeptide with strong amino acid sequence homology to the Tc^r proteins of pT181 (199). Other workers have reported molecular relatedness between pUB110 and Km^r plasmids from soil bacilli (197, 303, 326); significantly, the DNA sequence for the kanamycin-inactivating enzyme from one thermophile only differed from the sequence for AAD(4′)(4″) encoded on pUB110 by a single base, thereby resulting in a thermostable enzyme (303).

There is also support for the notion that some resistance determinants in staphylococci are derived from genes present in antibiotic-producing organisms. The *S. aureus ermC* methylase encoded on pE194 shares amino acid sequence homology with the analogous methylase encoded by erythromycin-producing organisms such as *Streptomyces erythraeus* (*ermE*) (522) and some *Arthrobacter* spp. (408). Notably, *ermD*, which encodes a methylase in *B. licheniformis* with partial amino acid sequence homology to the *ermC* and *ermE* methylases (181, 522), is flanked by sequences that are homologous with the *Streptomyces erythraeus* chromosome, thus invoking the possibility that MLS^r is encoded on a transposon in these soil microorganisms, from which it may have spread to staphylococci (213). Strong sequence homology has also been reported between the APH gene from the neomycin-producer *Streptomyces fradiae* and the APH(3′)III gene encoded by the *S. aureus* plasmid pSH2 (170), as well as with determinants for APH(3′)III encoded by transposons Tn*5* and Tn*903* from gram-negative species (494).

Substantially more evidence has been presented for the transfer of genetic material between *Streptococcus* spp. and *S. aureus*. The Tc^r determinants found on the *Streptococcus agalactiae* chromosome and on the *Streptococcus faecalis* transposon Tn*916* are homologous with a determinant carried by *S. aureus* (182), and the CAT of Cm^r strains of *Streptococcus agalactiae* cross-reacts immunologically with the staphylococcal CAT (559). Similarities have also been demonstrated between the aminoglycoside-modifying enzymes of both genera, including the APH(3′)III of *S. aureus* and those encoded by the chromosome of *Streptococcus pneumoniae* and a plasmid from *Streptococcus faecalis* (88, 514, 515). More recently, the AAC(6′)-APH(2″) polypeptides encoded by a self-transmissible plasmid from *S. faecalis* (91, 137a) and the *S. aureus* Gm^r Tm^r Km^r transposon Tn*4001* (296) have been shown to be identical (Rouch et al., submitted). Significantly, this homology, at the DNA sequence level, extended past the region encoding the C-terminal end of the AAC(6′)-APH(2″) polypeptide into sequences identical to $IS256_L$ on Tn*4001* (Fig. 11), thereby implying that this *S. faecalis* plasmid possesses Tn*4001* or a Tn*4001*-like element, perhaps equivalent to that on Gm^r plasmids isolated in the United States (Fig. 9 and 10) (292a).

The determinants of resistance to the MLS antibiotics in the streptococci are largely homologous with each other and in some cases with the staphylococcal MLS^r determinants (for a review, see reference 193); for example, the determinants in *Streptococcus pyogenes* and *Streptococcus pneumoniae* were shown by hybridization to be homologous with the determinant carried by the *S. aureus* transposon Tn*551* (535). Tn*551* and the similarly sized MLS^r *Streptococcus faecalis* transposon Tn*917* (451, 500) also exhibit extensive homology, not only throughout their lengths, as shown by restriction mapping and DNA heteroduplex analysis, but, importantly, in the sequence of their terminal inverted repeats (380); these repeats are also highly homologous with those of the gram-negative β-lactamase transposon Tn*3* (Table 5). All three elements generated 5-bp duplications of the target on insertion (231, 380). Whereas *ermB* of Tn*551* is constitutively expressed, *erm* of Tn*917* is induced by erythromycin, which also stimulates transposition of Tn*917* pos-

TABLE 5. Inverted repeat sequences of selected transposable elements from gram-positive and gram-negative bacteria

Element[a]		5'-Terminal sequence[b]	Reference
Tn*551* (J_L)	5'	GGGGTCCCGAGCGC* ACGAGAAATTTGTATCGATAAGAAATA	231
Tn*551* (J_R)	5'	GGGGTCCCGAGCGC* ACGAGAAATTTGTATCGATAAGGGGTA	231
Tn*917* (prox)	5'	GGGGTCCCGAGCGCCT ACGAGGAATTTGTATCGATAAGAAATA	380
Tn*917* (dist)	5'	GGGGTCCCGAGCGCTT AGTGGGAATTTGTATCGATAAGGGGTA	380
Tn*3*	5'	GGGGT CTGA CGC TC AGTGGAACGAAAACTCACGTTAAG	361
Tn*501*	5'	GGGGGAACCGCAGAATTCGGAAAAAATCGTACGCTAAG	50

[a] J_L and J_R, Left and right junctions of Tn*551* with pI258; prox and dist, proximal and distal ends of Tn*917* relative to the MLS gene.
[b] Asterisk denotes a proposed additional C residue not detected in the original sequence of Tn*551* (380).

sibly due to an extension of *erm*-specified transcription into the transposition genes (451). Tn*917*-like transposons appear to be widely distributed in *Streptococcus faecalis* strains (20, 411).

The transfer of MLS^r plasmids such as pAMβ1 from *Streptococcus* spp. to *S. aureus*, and subsequently between *S. aureus* strains, has been demonstrated (131, 433), but these plasmids have not been observed in natural isolates of staphylococci. Likewise, the streptococcal conjugative transposons Tn*916* (144), Tn*918* (76), and Tn*919* (140) have yet to be detected in *S. aureus*, although they are potentially useful for further genetic analysis of the staphylococcal chromosome (Fig. 1) (76, 76a, 373; P. A. Pattee, J. M. Jones, S. C. Yost, and J. E. Tam, *Genetic Maps*, in press). The intriguing observation that all *S. aureus* strains tested produced an extracellular peptide with a "pheromone" activity for conjugation with a *Streptococcus faecalis* donor harboring the conjugative plasmid pAM373, yet failed to establish this plasmid, prompted Clewell and co-workers (76) to suggest an alternative role for this peptide in *S. aureus*, viz., in virulence, since the activity was rarely produced by coagulase-negative strains.

Interspecific Transfer of Antibiotic Resistance Genes

It is now widely accepted that members of the genus *Staphylococcus* do not exist in genetic isolation and may in fact share certain plasmid pools. The similarity between Tc^r, Cm^r, and Nm^r plasmids isolated from strains of *S. epidermidis* and *S. aureus* was observed some time ago (415), and the Tc^r plasmids from both species have since been shown to be homologous (89, 173, 501; J. Tennent and R. Skurray, unpublished data), as have Cm^r plasmids (492, 492a). In the case of Em^r plasmids, there are now a number of reports of plasmids in *S. epidermidis* that are similar in size, but probably differ in overall structure, to those in *S. aureus* (141, 320, 335, 364). Of particular interest is the recent finding that the constitutive MLS^r determinant *ermM*, present on an *S. epidermidis* plasmid, is almost identical to the inducible *ermC* determinant on pE194, except for a deletion of sequences which would be necessary for translational attenuation (275).

The identity or close similarity of Gm^r plasmids found in epidemiologically related strains of *S. epidermidis* and *S. aureus* has also been demonstrated (79, 216, 217, 531), and there is homology between *S. aureus* and coagulase-negative strains with respect to cadmium (*cadA* and *cadB*) and penicillin (*blaZ*) resistance determinants (R. C. Cooksey and J. N. Baldwin, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 997, 1984). Homology between an arsenate resistance plasmid from *S. xylosus* and the *S. aureus* β-lactamase/heavy-metal resistance plasmid pI258 has also been shown (165). Further support for interspecific transfer of resistance determinants between *S. aureus* and *S. epidermidis* is provided by hybridization studies in this laboratory which demonstrated that clinical isolates of both species encoded antiseptic/disinfectant resistance via either *qacA* or *qacC*, Tp^r via *drfA*, and Gm^r via a Tn*4001*-encoded *aacA-aphD* (J. Tennent and R. Skurray, unpublished data); homologous Tp^r determinants in these species have also been detected by others (9). Enzymological studies have confirmed that high-level Tp^r strains of *S. epidermidis* which carry sequences homologous to *drfA* do indeed produce a DHFR with similar properties to the *S. aureus* type S1 enzyme (J. Tennent, B. Lyon, H.-K. Young, S. G. B. Amyes, and R. Skurray, submitted for publication).

The transfer of Pc^r and Cm^r plasmids in mixed cultures of *S. aureus* and *S. epidermidis* was the first indication that a mechanism of genetic transfer operates between different staphylococcal species (545). The transfer of Gm^r plasmids between *S. aureus* and *S. epidermidis* in vitro and on human skin has also been demonstrated (216), and a similar plasmid was transferred from a clinical isolate of *S. hominis* to several different *S. aureus* strains (338). In a few instances, the interspecific transfer of Gm^r plasmids was shown to have occurred by a conjugationlike process identical to that observed for intraspecific transfer in *S. aureus* (141, 308). The ability of these conjugal Gm^r plasmids to mobilize smaller plasmids, including those encoding Tc^r, Cm^r, and Em^r, from *S. epidermidis* to *S. aureus* (335) suggests a possible mechanism for the interspecific spread of other resistance determinants. There are also reports of in vitro (257) and in vivo (336) transfer of resistance determinants between human and animal staphylococci, although such exchange was considered by Lacey (257) to make little contribution to the *S. aureus* genetic pool.

Evolution of *S. aureus* Resistance Plasmids and the Multiresistant Chromosome

The evolution of antibiotic resistance plasmids in *S. aureus* is thought to occur by the same mechanisms of recombination that operate in other bacterial species. Whereas general recombination often involves the reciprocal exchange of DNA at regions of extensive homology, and requires the product of the bacterial *recA* gene, site-specific recombination of DNA segments can occur between sequences with little or no ancestral relationship, in the ab-

TABLE 6. *S. aureus* insertion sequences and transposons

Element	Resistance to[a]:	Size (kb)	Nature of terminal sequences	Source/location[b]	Reference(s)
Insertion sequences					
IS*256*		1.35		pSK1; chromosome	Lyon et al., submitted
IS*257* (IS*257*-like sequences)[c]		0.92[d]		pSK1; pSK74/pI524; chromosome associated with *mec*, *mer*, and *tet*	28; Gillespie et al., submitted; Matthews et al., submitted
Transposons					
Tn*551*	MLS	5.3	Inverted repeats of 40 bp	pI258	231, 350, 352, 354
Tn*552*	Pc	6.1		pI524; chromosome	332, 446
Tn*554*	MLS Sp	6.7	No repeated sequences	Chromosome	247, 330, 352, 384
Tn*3851*	Gm Tm Km	5.2		Chromosome	505
Tn*3852*	Pc	7.3		Chromosome	233
Tn*4001*	Gm Tm Km	4.7	Inverted repeats of IS*256*	pSK1; chromosome	157, 292a, 296, 297, 491; Rouch et al., submitted
Tn*4002*	Pc	6.7	Inverted repeats of ca. 80 bp	pSK4; chromosome	157; Gillespie et al., unpublished data
Tn*4003*[e]	Tp	3.6	Direct repeats of IS*257*	pSK1	Gillespie et al., submitted
Tn*4004*[c]	Hg	7.8	Direct repeats of IS*257*-like sequences	pSK74/pI524; chromosome	Gillespie et al., submitted
Tn*4201*	Pc	6.6	Inverted repeats of ca. 80 bp	pCRG1600, pUW3626	13; Weber and Goering, 25th ICAAC; Gillespie and Skurray, unpublished data
Tn*4291*	Mc	7.5		Chromosome	Trees and Iandolo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, and personal communication

[a] See Table 1, footnote *b*, for abbreviations of antimicrobial agents.
[b] Element detected on the *S. aureus* plasmid(s) described in the text or the *S. aureus* chromosome or both.
[c] The element and identical or highly homologous elements occur at more than one site in natural isolates but are yet to be shown to translocate in the laboratory.
[d] Although there is uncertainty as to the precise size of IS*257* and IS*257*-like sequences of pSK1 and pSK74/pI524, respectively, we have adopted the size (0.92 kb) evaluated for the IS*257*-like sequence present on the *S. aureus* chromosome in the region of *mec* (Matthews et al, submitted); it should be noted that one and possibly two of the four copies of the repeat present in the *mec* region may be truncated (Matthews and Stewart, personal communication).
[e] Tn*4003* is a tentative designation for this determinant based on its structural characteristics.

sence of the *recA* gene product (86). The latter mechanism thus provides for the quantal recombination (translocation or transposition) of "structurally defined" DNA segments which may have evolved in diverse genetic systems; a summary of staphylococcal translocatable sequences is provided in Table 6.

The characterization of families of *S. aureus* plasmids which mediate resistance to penicillin and heavy-metal ions (Fig. 2) (155, 157, 446), tobramycin and streptogramins (126), and gentamicin and other aminoglycosides (Fig. 9) (157, 169, 217) has revealed much information about the possible evolutionary pathways followed by individual plasmids. Members of each family were found to be largely homologous, but often possessed differences which could be explained by the deletion or insertion of discrete segments of DNA. Such differences could frequently be correlated with changes in plasmid phenotype and in some instances were believed to be the result of the activity of translocatable elements. Extensive plasmid deletions were also commonly observed following transduction (171, 308), thus implicating phage "packaging size" as a possible influence on plasmid evolution, and a potential brake on the development of more complex plasmids.

The recently emerged gentamicin resistance plasmids amply illustrate the part played by translocatable elements in staphylococcal plasmid evolution. In Australian isolates, Gm^r is encoded by the transposon Tn*4001* composed of inverted copies of IS*256* bounding the *aacA-aphD* determinant (Fig. 11). Tn*4001* was present at various chromosomal sites in the Gm^r strains isolated in Australia between 1977 and 1979 but thereafter was more commonly present on the pSK1 family of quaternary ammonium (*qacA*) resistance plasmids, thereby implicating chromosome to plasmid transposition. The *qacA* determinant, the feature common to all pSK1 family plasmids, is also encoded by β-lactamase/heavy-metal resistance plasmids, such as pSK57, and is closely related to the *qacB* determinant, present on plasmids detected as early as 1951 (Fig. 12; Gillespie and Skurray, unpublished data). These two antiseptic/disinfectant resistance determinants, which may differ by only a few nucleotides, could themselves represent translocatable sequences.

The pSK1 family of plasmids further enlarged its repertoire of resistance determinants at some stage by the acquisition of trimethoprim resistance via Tn*4003* and penicillin resistance through the insertion of Tn*4002* (Fig. 9 and 13). Elements of both Tn*4003* and Tn*4002* are present on β-lactamase/heavy-metal resistance plasmids; viz., direct repeats of IS*257* which bound the *dfrA* determinant on Tn*4003* are homologous with those which flank *mer* (Fig. 13), and it is highly likely that Tn*4002* evolved directly from such a plasmid (Fig. 3). Tn*4002* may well have integrated into the chromosome from an IncI group β-lactamase plasmid, under the pressure of incompatibility with another IncI group plasmid (506), and subsequently translocated to a pSK1 family plasmid to generate multiresistance plasmids, such as pSK4 (Fig. 9).

Gm^r plasmids in U.S. isolates, and possibly some in

European strains, although unrelated to the pSK1 family, have followed an analogous pathway of evolution, probably under the selective influence of similar antimicrobial agents in the environment. The Gm^r determinant on these plasmids appears entirely homologous to that of Tn*4001*, and the inverted repeats which bound it, although no longer able to direct transposition as we suspect IS*256* does, do have some homology with that element. It is of interest that Gm^r on the U.S. plasmids is, like Tn*4001* on the majority of pSK1 family plasmids, located immediately adjacent to a quaternary ammonium resistance determinant, although *qacC* on the U.S. plasmids is unrelated to *qacA* (Fig. 12); perhaps the sequences bounding *qacA* and *qacC* have provided preferential insertion sites for Tn*4001* and like elements. Some members of the U.S. Gm^r plasmid family have also gained Pc^r via Tn*4201*, which appears similar to Tn*4002* (Fig. 3), and Tp^r, which is encoded by an homologous *dfr* determinant to that on Tn*4003* and is also likely to be shown to be associated with repeat sequences similar or identical to IS*257*.

The most significant evolutionary event in the emergence of some U.S. Gm^r plasmids was the further acquisition of sequences which have rendered the plasmid self-transmissible. These *tra* genes must surely represent a highly refined sequence that had pre-evolved in another species or genus and was inserted in toto into a plasmid such as pSH6 to create Tra^+ plasmids such as pSK41 and pCRG1600 (Fig. 9). That all Tra^+ Gm^r plasmids examined also encoded Nm^r via an AAD(4′)(4″) determinant that lies adjacent to the *tra* region hints that the *aadD* and *tra* segments preexisted as a composite prior to insertion. It is tempting to speculate further that such a composite formed part of a conjugative transposon similar to those seen in the streptococci. The acquisition of these *tra* genes could be considered the final stage in the successful evolution of a multifactorial plasmid which is freed from constraints of size imposed by transduction and may continue to gain characteristics by transposition in a fashion akin to large multiresistance Tra^+ plasmids in gram-negative organisms.

An evolutionary progression similar to that seen for the Gm^r plasmids has been traced among the β-lactamase/heavy-metal resistance plasmids and, like the former, closely related, if not identical, plasmids of this type have demonstrated a wide geographical distribution (155, 157, 446). It is believed that the β-lactamase/heavy-metal resistance plasmids share common ancestry due to the existence of highly conserved regions encompassing *mer-cadA* and *asa–asi-ant* (Fig. 2; 446). Major molecular rearrangements seem to have been restricted to the region lying between *cadA* and the mcr-encoding region, indicating that this may represent a "hot spot" for genetic reorganization (Gillespie and Skurray, unpublished data). A hypothetical pathway for the evolution of the β-lactamase/heavy-metal resistance plasmids suggests that they may have derived from a small Cd^r plasmid, such as pIP983 (Table 2), by successive transposition and recombination events (446); a similar series of events could be theorized for the evolution of the U.S. Gm^r plasmids from a small Eb^r Qa^r plasmid such as pSK89 (Table 3).

An alternative mechanism to transposon insertion in the evolution of plasmid complexity has been suggested by the formation of apparently irreversible cointegrates between small staphylococcal plasmids (212, 351). Such interactions allude to a modular organization for these plasmids (352), perhaps not unlike that seen in the cointegrate plasmids of gram-negative organisms (86). Some of the sequences at which cointegrate formation occurs have been identified on a number of small plasmids. The recombination site RS_A (ca. 70 bp) is present on pT181 and pE194, and RS_B (ca. 30 bp) is also present on these two plasmids as well as on pC194, pC221, pS194, and pUB110 (Tables 3 and 4) (357, 394). Cointegration of pT181 and pC221 also occurred by crossovers between homologous sequences shared by the C-terminal regions of *repC* and *repD* of these plasmids and regions of dyad symmetry located in the *tet* and *cat* terminators (Fig. 7 and 8) (394). It is highly likely that sequences such as RS_B and the *cat* terminator have also been responsible for the preservation/distribution of *cat* and *repD* sequences among the pC221 family of Cm^r plasmids and could well be involved in the evolution of the pC194 family (Fig. 7; Gillespie and Skurray, unpublished data). It will be of some interest to establish if RS are present on larger staphylococcal plasmids and have, through recombination, contributed to their multiresistant nature.

The mechanisms of site-specific and generalized recombination have also played a central role in the evolution of a multiresistant chromosome, a feature of *S. aureus* strains currently endemic in many hospitals throughout the world (44, 87, 125, 293, 295). A retrospective analysis of *S. aureus* isolates collected at two Melbourne hospitals since 1946 has permitted some insights into the progressive accumulation of chromosomal resistance determinants (153, 154; Gillespie and Skurray, unpublished data). Significantly, a number of these determinants in the "epidemic" strain(s), including those for Tc^r, Pc^r, and Hg^r, are homologous with determinants which were plasmid encoded in isolates prior to 1970. In some instances there is evidence for transposition from plasmid to chromosome, as in the case of Pc^r (Tn*4002*) and Hg^r (Tn*4004*), which were originally detected on β-lactamase/heavy-metal resistance plasmids. On the other hand, integration of an entire Tc^r plasmid is seen in the case of pT181 which presumably underwent generalized recombination with the chromosome at the int site (Fig. 8).

Despite the mechanism, chromosomal acquisition of some resistance characters may have only occurred on a single occasion. This idea is supported by the observations that Tn*4002* (Pc^r) and pT181 (Tc^r) each occupied a common site in all strains tested (157; Gillespie and Skurray, unpublished data; Jones and Pattee, personal communication). Such evidence also supports the notion that many Mc^r strains have a common origin (223, 255) and that the "new" multiresistant hospital staphylococci are in fact "old" staphylococci that have acquired additional chromosomal resistance determinants by integration as well as by mutation, as in the case of rifampin and fusidic acid resistances (149, 259, 506). That Gm^r occurs at a variety of sites on the chromosomes of Mc^r clinical isolates (Gillespie et al., J. Med. Microbiol., in press) argues for a higher natural transposition frequency for Tn*4001* compared with some other elements and for insertion into an Mc^r strain that had previously acquired chromosomal Pc^r, Hg^r, and Tc^r.

The exact roles of insertion sequences in providing regions of homology for integration and subsequent evolution of the multiresistant chromosome await detailed investigation. For example, IS*256* sequences have been detected at multiple sites on the chromosomes of some Mc^r Gm^s strains (Lyon et al., submitted) and such could represent either single insertions, which can result in genetic rearrangement of the neighboring genes (201), or unidentified transposons. IS*256* may thus provide a crucial component for *S. aureus* strains to fabricate new composite transposons carrying preexisting chromosomal (or plasmid) resistance or virulence determi-

nants. In contrast to IS*256*, IS*257* sequences occur at limited sites on the chromosome and so far only in conjunction with the determinants for methicillin, mercury, and tetracycline resistance (28; Matthews et al., submitted; Gillespie, et al., submitted). It will be of considerable interest to establish the detailed molecular organization and DNA sequence of the putative *mec-mer-tet* cluster on the chromosome and the role played by IS*257*- or IS*257*-like sequences in the establishment and evolution of the enigmatic methicillin resistance transposon.

CONCLUDING REMARKS

It has frequently been argued that extensive, and often indiscriminate, use of antimicrobial agents has produced sufficiently high levels of these compounds in the hospital environment to act as selective pressures for the de novo creation or acquisition of resistance determinants. This is no doubt the case with many staphylococcal resistance determinants, since emergence of resistance frequently followed the introduction of the agent. There are, however, cases of determinants being detected in isolates which predate the introduction of an antimicrobial agent, and resistance to penicillin via a β-lactamase (367, 388) and fusidic acid resistance (154) are two examples. Regardless of when they evolved, the spread of resistance characters among the staphylococcal population and the survival of newly resistant variants is most certainly promoted by environmental antimicrobial agents.

One may also consider the selective pressures that have contributed to the maintenance of particular plasmids, often at the apparent expense of others. For example, the contemporary *S. aureus* strains endemic in many hospitals worldwide, possess plasmids such as the pSK1 family or the self-transmissible Gm^r plasmids, which share resistance to antiseptics and disinfectants as well as also encoding Tp^r and Pc^r in some instances (Table 4). What pressures led to the predominance of these plasmids over the formerly prevalent β-lactamase/heavy-metal resistance plasmids? Was resistance to hospital antiseptics and disinfectants, such as benzalkonium chloride, cetrimide, and propamidine isethionate, the raison d'etre for their maintenance and spread or do they possess inherent replicative advantages compared to β-lactamase plasmids? There may well be other properties encoded by these plasmids, the maps of which are far from genetic saturation, which endow their hosts with a selective advantage. For example, it is not known if any of the plasmids carried by these contemporary strains, including the small phenotypically cryptic plasmids (293), influence their host's apparent propensity to colonize patients; it may be noted that these strains do not show enhanced resistance to either linolenic acid (307), a component of skin lipid which is inhibitory for *S. aureus* (267), or desiccation (149). Further, in the development of a multiresistant chromosome, it is not clear what environmental pressures resulted in the chromosome gaining of such elements as a Tc^r plasmid and the Pc^r and Hg^r determinants, which were commonly located on β-lactamase/heavy-metal resistance plasmids. Obviously, such integration provides one advantage by "making space" for the acquisition of new characteristics that are encoded on plasmids of the same incompatibility group as the erstwhile resident plasmid.

This review is not the venue for a detailed discussion of the most effective and new approaches to antistaphylococcal therapy (see references 65, 236, 264, 490, 527a, and 541; Editorial, Lancet **ii**:189–190, 1985) or of the measures to control outbreaks of nosocomial infection. Suffice it to say that hospital microbiologists and infection control staff should, in their quest to reduce the impact of these organisms in human and economic terms, not only consider the use of isolation units, appropriate antiseptics/disinfectants, and the adoption of stringent hygienic practices in the care of infected and colonized patients (44, 63, 69a, 377, 448a, 449; Editorial, Lancet **ii**:189–190, 1985), but also look to the education of hospital staff in the factors and mechanisms which may result in the emergence and spread of resistant staphylococci and to the adoption and enforcement of strict codes of usage for antimicrobial agents (274, 309). Furthermore, the application of modern molecular biological and immunological techniques to the development of more rapid procedures for the identification of multiresistant *S. aureus* and the determination of their resistance profiles will, undoubtedly, lead to a reduction in the abuse and misuse of valuable antimicrobial agents. Similar molecular techniques should also gain wider acceptance in epidemiological studies of nosocomial staphylococcal strains and the resistance determinants, plasmids, and transposons that they harbor. We hope that this review will provide some stimulus towards increased activity in these areas.

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LITERATURE CITED

1. **Alonso, J. C., and T. A. Trautner.** 1985. A gene controlling segregation of the *Bacillus subtilis* plasmid pC194. Mol. Gen. Genet. **198**:427–431.
2. **Altboum, Z., I. Hertman, and S. Sarid.** 1985. Penicillinase plasmid-linked genetic determinants for enterotoxins B and C_1 production in *Staphylococcus aureus*. Infect. Immun. **47**:514–521.
3. **Ambler, R. P.** 1975. The amino acid sequence of the *Staphylococcus aureus* penicillinase. Biochem. J. **151**:197–218.
4. **Ambulos, N. P., Jr., J. H. Chow, S. Mongkolsuk, L. H. Preis, W. R. Vollmar II, and P. S. Lovett.** 1984. Constitutive variants of the pC194 *cat* gene exhibit DNA alterations in the vicinity of the ribosome binding site sequence. Gene **28**:171–176.
5. **Annear, D. I.** 1968. The effect of temperature on resistance of *Staphylococcus aureus* to methicillin and some antibiotics. Med. J. Aust. **1**:444–446.
6. **Annear, D. I., and W. B. Grubb.** 1972. Unstable resistance to kanamycin, lincomycin and penicillin in a methicillin resistant culture of *Staphylococcus aureus*. Pathology **4**:247–252.
7. **Annear, D. I., and W. B. Grubb.** 1976. Methicillin-sensitive variants in ageing broth-cultures of methicillin-resistant *Staphylococcus aureus*. Pathology **8**:69–72.
8. **Archer, G. L.** 1984. *Staphylococcus epidermidis*: the organism, its diseases, and treatment, p. 25–48. *In* J. S. Remington and M. N. Swartz (ed.), Current clinical topics in infectious diseases, vol. 5. McGraw-Hill Book Co., New York.
9. **Archer, G. L., J. P. Coughter, and J. L. Johnston.** 1986. Plasmid-encoded trimethoprim resistance in staphylococci. Antimicrob. Agents Chemother. **29**:733–740.
10. **Archer, G. L., D. R. Dietrick, and J. L. Johnston.** 1985. Molecular epidemiology of transmissible gentamicin resistance

among coagulase-negative staphylococci in a cardiac surgery unit. J. Infect. Dis. **151**:243–251.
11. **Archer, G. L., and J. L. Johnston.** 1983. Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. Antimicrob. Agents Chemother. **24**:70–77.
12. **Archer, G. L., and C. G. Mayhall.** 1983. Comparison of epidemiological markers used in the investigation of an outbreak of methicillin-resistant *Staphylococcus aureus* infections. J. Clin. Microbiol. **18**:395–399.
13. **Asch, D. K., R. V. Goering, and E. A. Ruff.** 1984. Isolation and preliminary characterization of a plasmid mutant derepressed for conjugal transfer in *Staphylococcus aureus*. Plasmid **12**:197–202.
14. **Asheshov, E. H.** 1966. Chromosomal location of the genetic elements controlling penicillinase production in a strain of *Staphylococcus aureus*. Nature (London) **210**:804–806.
15. **Asheshov, E. H.** 1969. The genetics of penicillinase production in *Staphylococcus aureus* strain PS80. J. Gen. Microbiol. **59**:289–301.
16. **Asheshov, E. H.** 1975. The genetics of tetracycline resistance in *Staphylococcus aureus*. J. Gen. Microbiol. **88**:132–140.
17. **Ayliffe, G. A. J., W. Green, R. Livingston, and E. J. L. Lowbury.** 1977. Antibiotic-resistant *Staphylococcus aureus* in dermatology and burns wards. J. Clin. Pathol. **30**:40–44.
18. **Bächi, B.** 1980. Physical mapping of *Bgl*I, *Bgl*II, *Pst*I and *Eco*RI restriction fragments of staphylococcal phage φ11 DNA. Mol. Gen. Genet. **180**:391–398.
19. **Baird-Parker, A. C.** 1972. Classification and identification of staphylococci and their resistance to physical agents, p. 1–20. *In* J. O. Cohen (ed.), The staphylococci. John Wiley & Sons, Inc., New York.
20. **Banai, M., and D. J. LeBlanc.** 1984. *Streptococcus faecalis* R plasmid pJH1 contains an erythromycin resistance transposon (Tn*3871*) similar to transposon Tn*917*. J. Bacteriol. **158**:1172–1174.
21. **Barber, M.** 1947. Staphylococcal infection due to penicillin-resistant strains. Br. Med. J. **2**:863–865.
22. **Barber, M.** 1949. The incidence of penicillin-sensitive variant colonies in penicillinase-producing strains of *Staphylococcus pyogenes*. J. Gen. Microbiol. **3**:274.
23. **Barber, M., and M. Rozwadowska-Dowzenko.** 1948. Infection by penicillin-resistant staphylococci. Lancet **ii**:641–644.
24. **Barna, J. C. J., and D. H. Williams.** 1984. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Annu. Rev. Microbiol. **38**:339–357.
24a. **Barr, V., K. Barr, M. R. Millar, and R. W. Lacey.** 1986. β-Lactam antibiotics increase the frequency of plasmid transfer in *Staphylococcus aureus*. J. Antimicrob. Chemother. **17**:409–413.
25. **Barrett, F. F., R. F. McGehee, Jr., and M. Finland.** 1968. Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital: bacteriologic and epidemiologic observations. N. Engl. J. Med. **279**:441–448.
26. **Bastos, M. C. F., M. C. Bonaldo, and E. G. C. Penido.** 1980. Constitutive erythromycin resistance plasmid in *Staphylococcus aureus*. J. Gen. Microbiol. **121**:513–516.
27. **Beard-Pegler, M. A., and A. M. Vickery.** 1985. Lysogenicity of methicillin-resistant strains of *Staphylococcus aureus*. J. Med. Microbiol. **20**:147–155.
28. **Beck, W. D., B. Berger-Bächi, and F. H. Kayser.** 1986. Additional DNA in methicillin-resistant *Staphylococcus aureus* and molecular cloning of *mec*-specific DNA. J. Bacteriol. **165**:373–378.
29. **Bennett, P. M., and M. H. Richmond.** 1978. Plasmids and their possible influence on bacterial evolution, p. 1–69. *In* I. C. Gunsalus, L. N. Ornston, and J. R. Sokatch (ed.), The bacteria, vol. 6. Academic Press, Inc., New York.
30. **Berger-Bächi, B.** 1983. Insertional inactivation of staphylococcal methicillin resistance by Tn*551*. J. Bacteriol. **154**:479–487.
31. **Berger-Bächi, B.** 1983. Increase in transduction efficiency of Tn*551* mediated by the methicillin resistance marker. J. Bacteriol. **154**:533–535.
32. **Berger-Bächi, B., and M. L. Kohler.** 1983. A novel site on the chromosome of *Staphylococcus aureus* influencing the level of methicillin resistance: genetic mapping. FEMS Microbiol. Lett. **20**:305–309.
33. **Bernhard, K., H. Schrempf, and W. Goebel.** 1978. Bacteriocin and antibiotic resistance plasmids in *Bacillus cereus* and *Bacillus subtilis*. J. Bacteriol. **133**:897–903.
34. **Berryhill, D. L., and P. A. Pattee.** 1969. Buoyant density analysis of staphylococcal bacteriophage 80 transducing particles. J. Virol. **4**:804–806.
35. **Best, G. K., N. H. Best, and A. V. Koval.** 1974. Evidence for participation of autolysins in bactericidal action of oxacillin on *Staphylococcus aureus*. Antimicrob. Agents Chemother. **6**:825–830.
36. **Bingham, A. H. A., T. Atkinson, and C. J. Bruton.** 1982. Construction and characterization of a chimeric plasmid in *Bacillus subtilis*. Plasmid **8**:119–125.
37. **Bingham, A. H. A., C. J. Bruton, and T. Atkinson.** 1979. Isolation and partial characterization of four plasmids from antibiotic-resistant thermophilic bacilli. J. Gen. Microbiol. **114**:401–408.
38. **Bint, A. J., R. H. George, D. E. Healing, R. Wise, and M. Davies.** 1977. An outbreak of infection caused by a gentamicin-resistant *Staphylococcus aureus*. J. Clin. Pathol. **30**:165–167.
39. **Birmingham, V. A., and P. A. Pattee.** 1981. Genetic transformation in *Staphylococcus aureus*: isolation and characterization of a competence-conferring factor from bacteriophage 80α lysates. J. Bacteriol. **148**:301–307.
40. **Blanchard, T. J., S. M. Poston, and P. J. Reynolds.** 1986. Recipient characteristics in the transduction of methicillin resistance in *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **29**:539–541.
41. **Borowski, J., K. Kamieńska, and I. Rutecka.** 1964. Methicillin-resistant staphylococci. Br. Med. J. **1**:983.
42. **Boyce, J. M., R. L. White, W. A. Causey, and W. R. Lockwood.** 1983. Burn units as a source of methicillin-resistant *Staphylococcus aureus* infections. J. Am. Med. Assoc. **249**:2803–2807.
43. **Bradley, H. E., J. G. Wetmur, and D. S. Hodes.** 1980. Tolerance in *Staphylococcus aureus*: evidence for bacteriophage role. J. Infect. Dis. **141**:233–237.
44. **Bradley, J. M., P. Noone, D. E. Townsend, and W. B. Grubb.** 1985. Methicillin-resistant *Staphylococcus aureus* in a London hospital. Lancet **i**:1493–1495.
45. **Brenner, D. G., and W. V. Shaw.** 1985. The use of synthetic oligonucleotides with universal templates for rapid DNA sequencing: results with staphylococcal replicon pC221. EMBO J. **4**:561–568.
46. **Broda, P.** 1979. Plasmids. W. H. Freeman Co., San Francisco.
47. **Bronson, D. L., and P. A. Pattee.** 1972. Genetic analysis of resistance to erythromycin and oleandomycin in *Staphylococcus aureus*. Can. J. Microbiol. **18**:429–434.
48. **Brown, D. F. J., and P. E. Reynolds.** 1980. Intrinsic resistance to β-lactam antibiotics in *Staphylococcus aureus*. FEBS Lett. **122**:275–278.
49. **Brown, D. F. J., and P. E. Reynolds.** 1983. Transpeptidation in *Staphylococcus aureus* with intrinsic resistance to β-lactam antibiotics ("methicillin resistance"), p. 537–542. *In* R. Hackenbeck, J.-V. Höltje, and H. Labischinski (ed.), The target of penicillin. Walter de Gruyter, Berlin.
50. **Brown, N. L., C.-L. Choi, J. Grinsted, M. H. Richmond, and P. R. Whitehead.** 1980. Nucleotide sequences at the ends of the mercury resistance transposon Tn*501*. Nucleic Acids Res. **8**:1933–1945.
51. **Brückner, R., and H. Matzura.** 1985. Regulation of the inducible chloramphenicol acetyltransferase gene of the *Staphylococcus aureus* plasmid pUB112. EMBO J. **4**:2295–2300.
52. **Brückner, R., E. Zyprian, and H. Matzura.** 1984. Expression of a chloramphenicol-resistance determinant carried on hybrid plasmids in Gram-positive and Gram-negative bacteria. Gene **32**:151–160.
53. **Brumfitt, W., S. Dixson, and J. M. T. Hamilton-Miller.** 1985. Resistance to antiseptics in methicillin and gentamicin resistant *Staphylococcus aureus*. Lancet **i**:1442–1443.
54. **Bruns, W., H. Keppeler, and R. Baucks.** 1985. Suppression of

intrinsic resistance to penicillins in *Staphylococcus aureus* by polidocanol, a dodecyl polyethyleneoxid ether. Antimicrob. Agents Chemother. **27**:632–639.
55. **Brunton, J.** 1984. Antibiotic resistance plasmids of streptococci, staphylococci, and bacteroides, p. 529–565. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
56. **Bryan, L. E.** 1984. Aminoglycoside resistance, p. 241–277. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
57. **Bryan, L. E., and S. Kwan.** 1983. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. Antimicrob. Agents Chemother. **23**:835–845.
58. **Buckwold, F. J., W. L. Albritton, A. R. Ronald, J. Lertzman, and R. Henriksen.** 1979. Investigations of the occurrence of gentamicin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **15**:152–156.
59. **Bulger, R., and J. C. Sherris.** 1968. Decreased incidence of antibiotic resistance among *Staphylococcus aureus*: a study in a university hospital over a nine year period. Ann. Intern. Med. **69**:1099–1108.
60. **Bulow, P.** 1971. Staphylococci in Danish hospitals during the last decade: factors influencing some properties of predominant epidemic strains. Ann. N.Y. Acad. Sci. **182**:21–39.
61. **Burchall, J. J., L. P. Elwell, and M. E. Fling.** 1982. Molecular mechanisms of resistance to trimethoprim. Rev. Infect. Dis. **4**:246–254.
62. **Byeon, W.-H., and B. Weisblum.** 1984. Post-transcriptional regulation of chloramphenicol acetyltransferase. J.Bacteriol. **158**:543–550.
63. **Cafferkey, M. T., R. Hone, D. Coleman, H. Pomeroy, B. McGrath, R. Ruddy, and C. T. Keane.** 1985. Methicillin-resistant *Staphylococcus aureus* in Dublin 1971–1984. Lancet **ii**:705–708.
64. **Cafferkey, M. T., R. Hone, F. R. Falkiner, C. T. Keane, and H. Pomeroy.** 1983. Gentamicin and methicillin resistant *Staphylococcus aureus* in Dublin hospitals: clinical and laboratory studies. J. Med. Microbiol. **16**:117–127.
65. **Cafferkey, M. T., R. Hone, and C. T. Keane.** 1982. Severe staphylococcal infections treated with vancomycin. J. Antimicrob. Chemother. **9**:69–74.
66. **Canawati, H. N., J. L. Witte, and F. L. Sapico.** 1982. Temperature effect on the susceptibility of methicillin-resistant *Staphylococcus aureus* to four different cephalosporins. Antimicrob. Agents Chemother. **21**:173–175.
67. **Canepari, P., P. E. Varaldo, R. Fontana, and G. Satta.** 1985. Different staphylococcal species contain various numbers of penicillin-binding proteins ranging from four (*Staphylococcus aureus*) to only one (*Staphylococcus hyicus*). J. Bacteriol. **163**:796–798.
68. **Canosi, U., G. Morelli, and T. A. Trautner.** 1978. The relationship between molecular structure and transformation efficiency of some *S. aureus* plasmids isolated from *B. subtilis*. Mol. Gen. Genet. **166**:259–267.
69. **Carleton, S., S. J. Projan, S. K. Highlander, S. M. Moghazeh, and R. P. Novick.** 1984. Control of pT181 replication. II. Mutational analysis. EMBO J. **3**:2407–2414.
69a. **Casewell, M. W.** 1986. Epidemiology and control of the 'modern' methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **7**(Suppl. A):1–11.
70. **Chambers, H. F., B. J. Hartman, and A. Tomasz.** 1985. Increased amounts of a novel penicillin-binding protein in a strain of methicillin-resistant *Staphylococcus aureus* exposed to nafcillin. J. Clin. Invest. **76**:325–331.
71. **Chopra, I.** 1976. Mechanisms of resistance to fusidic acid in *Staphylococcus aureus*. J. Gen. Microbiol. **96**:229–238.
72. **Chopra, I., P. M. Bennett, and R. W. Lacey.** 1973. A variety of staphylococcal plasmids present as multiple copies. J. Gen. Microbiol. **79**:343–345.
73. **Chopra, I., and T. G. B. Howe.** 1978. Bacterial resistance to the tetracyclines. Microbiol. Rev. **42**:707–724.
74. **Chopra, I., R. W. Lacey, and J. Connolly.** 1974. Biochemical and genetic basis of tetracycline resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **6**:397–404.
75. **Clewell, D. B.** 1981. Plasmids, drug resistance, and gene transfer in the genus *Streptococcus*. Microbiol. Rev. **45**:409–436.
76. **Clewell, D. B., F. Y. An, B. A. White, and C. Gawron-Burke.** 1985. *Streptococcus faecalis* sex pheromone (cAM373) also produced by *Staphylococcus aureus* and identification of a conjugative transposon (Tn*918*). J. Bacteriol. **162**:1212–1220.
76a. **Clewell, D. B., and C. Gawron-Burke.** 1986. Conjugative transposons and the dissemination of antibiotic resistance in streptococci. Annu. Rev. Microbiol. **40**:635–659.
77. **Clewell, D. B., B. A. White, Y. Ike, and F. Y. An.** 1984. Sex pheromones and plasmid transfer in *Streptococcus faecalis*, p. 133–149. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
78. **Clowes, R. C.** 1972. Molecular structure of bacterial plasmids. Bacteriol. Rev. **36**:361–405.
79. **Cohen, M. L., E. S. Wong, and S. Falkow.** 1982. Common R-plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis* during a nosocomial *Staphylococcus aureus* outbreak. Antimicrob. Agents Chemother. **21**:210–215.
80. **Cohen, S., and H. M. Sweeney.** 1968. Constitutive penicillinase formation in *Staphylococcus aureus* owing to a mutation unlinked to the penicillinase plasmid. J. Bacteriol. **95**: 1368–1374.
81. **Cohen, S., and H. M. Sweeney.** 1970. Transduction of methicillin resistance in *Staphylococcus aureus* dependent on an unusual specificity of the recipient strain. J. Bacteriol. **104**:1158–1167.
82. **Cohen, S., and H. M. Sweeney.** 1973. Effect of the prophage and penicillinase plasmid of the recipient strain upon the transduction and the stability of methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **116**:803–811.
83. **Cohen, S., and H. M. Sweeney.** 1979. Modulation of protein A formation in *Staphylococcus aureus* by genetic determinants for methicillin resistance. J. Bacteriol. **140**:1028–1035.
84. **Cohen, S., H. M. Sweeney, and S. K. Basu.** 1977. Mutations in prophage ϕ11 that impair the transducibility of their *Staphylococcus aureus* lysogens for methicillin resistance. J. Bacteriol. **129**:237–245.
85. **Cohen, S., E. G. Vernon, and H. M. Sweeney.** 1970. Differential depression of staphylococcal plasmid and chromosomal penicillinase genes by a class of unlinked chromosomal mutations ($R2^-$). J. Bacteriol. **103**:616–621.
86. **Cohen, S. N.** 1976. Transposable genetic elements and plasmid evolution. Nature (London) **263**:731–738.
87. **Coleman, D. C., H. Pomeroy, J. K. Estridge, C. T. Keane, M. T. Cafferkey, R. Hone, and T. J. Foster.** 1985. Susceptibility to antimicrobial agents and analysis of plasmids in gentamicin- and methicillin- resistant *Staphylococcus aureus* from Dublin hospitals. J. Med. Microbiol. **20**:157–167.
88. **Collatz, E., C. Carlier, and P. Courvalin.** 1983. The chromosomal 3′, 5″-aminoglycoside phosphotransferase in *Streptococcus pneumoniae* is closely related to its plasmid-coded homologs in *Streptococcus faecalis* and *Staphylococcus aureus*. J. Bacteriol. **156**:1373–1377.
89. **Cooksey, R. C., and J. N. Baldwin.** 1985. Relatedness of tetracycline resistance plasmids among species of coagulase-negative staphylococci. Antimicrob. Agents Chemother. **27**:234–238.
90. **Courtieu, A. L., F. N. Guillermet, C. Longeray, G. Maka, and Y. A. Chabbert.** 1964. Fréquence des staphylocoques présentant une résistance hétérogène à la methicilline et l'oxacilline en milliéu hospitalier. Ann. Inst. Pasteur (Paris) **107**:691–697.
91. **Courvalin, P., C. Carlier, and E. Collatz.** 1980. Plasmid-mediated resistance to aminocyclitol antibiotics in group D streptococci. J. Bacteriol. **143**:541–551.
92. **Courvalin, P., and M. Fiandt.** 1980. Aminoglycoside-modifying enzymes of *Staphylococcus aureus*: expression in *Escherichia coli*. Gene **9**:247–269.
93. **Courvalin, P., H. Ounissi, and M. Arthur.** 1985. Multiplicity of

macrolide-lincosamide-streptogramin antibiotic resistance determinants. J. Antimicrob. Chemother. **16**(Suppl. A):91–100.

94. **Craven, D. E., C. Reed, N. Kollisch, A. DeMaria, D. Lichtenberg, K. Shen, and W. R. McCabe.** 1981. A large outbreak of infections caused by a strain of *Staphylococcus aureus* resistant to oxacillin and aminoglycosides. Am. J. Med. **71**:53–58.
95. **Crossley, K., D. Loesch, B. Landesman, K. Mead, M. Chern, and R. Strate.** 1979. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. I. Clinical studies. J. Infect. Dis. **139**:273–279.
96. **Cundliffe, E.** 1984. Self defence in antibiotic-producing organisms. Br. Med. Bull. **40**:61–67.
97. **Curtis, N. A. C., and M. V. Hayes.** 1981. A mutant of *Staphylococcus aureus* H deficient in penicillin-binding protein 1 is viable. FEMS Microbiol. Lett. **10**:227–229.
98. **Curtis, N. A. C., M. V. Hayes, A. W. Wyke, and J. B. Ward.** 1980. A mutant of *Staphylococcus aureus* H lacking penicillin-binding protein 4 and transpeptidase activity in vitro. FEMS Microbiol. Lett. **9**:263–266.
99. **Dagert, M., I. Jones, A. Goze, S. Romac, B. Niaudet, and S. D. Ehrlich.** 1984. Replication functions of pC194 are ncessary for efficient plasmid transduction by M13 phage. EMBO J. **3**:81–86.
100. **Damper, P. D., and W. Epstein.** 1981. Role of the membrane potential in bacterial resistance to aminoglycoside antibiotics. Antimicrob. Agents Chemother. **20**:803–808.
101. **Davies, J., and D. I. Smith.** 1978. Plasmid-determined resistance to antimicrobial agents. Annu. Rev. Microbiol. **32**:469–518.

101a. **Davis, B. D., L. Chen, and P. C. Tai.** 1986. Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. Proc. Natl. Acad. Sci. USA **83**:6164–6168.

102. **Della Latta, P., D. Bouarlchaud, and R. P. Novick.** 1978. Partition kinetics and thermosensitive replication of pT169, a naturally occurring multicopy tetracycline resistance plasmid of *Staphylococcus aureus*. Plasmid **1**:366–375.
103. **De Meester, C., and J. Rondelet.** 1976. Microbial acetylation of M factor of virginiamycin. J. Antibiot. **29**:1297–1305.
104. **De Saxe, M., and A. Porthouse.** 1979. Gentamicin resistance in *Staphylococcus aureus*. Lancet **ii**:370–371.
105. **Docherty, A., G. Grandi, R. Grandi, T. J. Gryczan, A. G. Shivakumar, and D. Dubnau.** 1981. Naturally occurring macrolide-lincosamide-streptogramin B resistance in *Bacillus licheniformis*. J. Bacteriol. **145**:129–137.
106. **Dornbusch, K., and H. O. Hallander.** 1973. Transduction of penicillinase production and methicillin resistance-enterotoxin B production in strains of *Staphylococcus aureus*. J. Gen. Microbiol. **76**:1–11.
107. **Dornbusch, K., H. O. Hallander, and F. Löfquist.** 1969. Extrachromosomal control of methicillin resistance and toxin production in *Staphylococcus aureus*. J. Bacteriol. **98**:351–358.
108. **Dowd, G., M. Cafferkey, and G. Dougan.** 1983. Gentamicin and methicillin resistant *Staphylococcus aureus* in Dublin hospitals: molecular studies. J. Med. Microbiol. **16**:129–138.
109. **Dubnau, D.** 1984. Translational attenuation: the regulation of bacterial resistance to the macrolide-lincosamide-streptogramin B antibiotics. Crit. Rev. Biochem. **16**:103–132.
110. **Dubnau, D.** 1985. Induction of *ermC* requires translation of the leader peptide. EMBO J. **4**:533–537.
111. **Dyer, D. W., M. I. Rock, Y. L. Chia, and J. J. Iandolo.** 1985. Generation of transducing particles in *Staphylococcus aureus*. J. Bacteriol. **161**:91–95.
112. **Dyer, D. W., M. I. Rock, and J. J. Iandolo.** 1984. Autogenous transduction of φ11de in *Staphylococcus aureus*: transfer and genetic properties. J. Bacteriol. **158**:689–695.
113. **Dyke, K. G. H.** 1969. Penicillinase production and intrinsic resistance to penicillins in methicillin-resistant cultures of *Staphylococcus aureus*. J. Med. Microbiol. **2**:261–278.
114. **Dyke, K. G. H.** 1979. β-Lactamases of *Staphylococcus aureus*, p. 291–310. *In* J. M. T. Hamilton-Miller and J. T. Smith (ed.), Beta-lactamases. Academic Press, Inc. (London), Ltd., London.
115. **Dyke, K. G. H., and W. C. Noble.** 1984. Plasmids of phage-group-II *Staphylococcus aureus*. J. Med. Microbiol. **17**:325–334.
116. **Dyke, K. G. H., M. T. Parker, and M. H. Richmond.** 1970. Penicillinase production and metal-ion resistance in *Staphylococcus aureus* cultures isolated from hospital patients. J. Med. Microbiol. **3**:125–136.
117. **Easmon, C. S. F., and C. Adlam.** 1983. Staphylococci and staphylococcal infections, vol. 1. Clinical and epidemiological aspects. Academic Press, Inc. (London), Ltd., London.
118. **Easmon, C. S. F., and C. Adlam.** 1983. Staphylococci and staphylococcal infections, vol. 2. The organism *in vivo* and *in vitro*. Academic Press, Inc. (London), Ltd., London.
119. **Ehrlich, S. D.** 1977. Replication and expression of plasmids from *Staphylococcus aureus* in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **74**:1680–1682.
120. **Ehrlich, S. D., B. Niaudet, and B. Michel.** 1982. Use of plasmids from *Staphylococcus aureus* for cloning of DNA in *Bacillus subtilis*. Curr. Top. Microbiol. Immunol. **96**:19–29.
121. **Eisenberg, E. S., L. J. Mandel, H. R. Kaback, and M. H. Miller.** 1984. Quantitative association between electrical potential across the cytoplasmic membrane and early gentamicin uptake and killing in *Staphylococcus aureus*. J. Bacteriol. **157**:863–867.
122. **El Nima, E. I.** 1984. The synergism between cetrimide and antibiotics against *Pseudomonas aeruginosa*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **258**:120–127.
123. **El Solh, N., R. Bismuth, J. Allignet, and J. M. Fouace.** 1984. Résistance à la pristinamycine (ou virginiamycine) des souches de *Staphylococcus aureus*. Pathol. Biol. **32**:362–368.
124. **El Solh, N., and S. D. Ehrlich.** 1982. A small cadmium resistance plasmid isolated from *Staphylococcus aureus*. Plasmid **7**:77–84.
125. **El Solh, N., J. M. Fouace, J. Pillet, and Y. A. Chabbert.** 1981. Plasmid DNA content of multiresistant *Staphylococcus aureus* strains. Ann. Microbiol. (Paris) **132B**:131–156.
126. **El Solh, N., J. M. Fouace, Z. Shalita, D. H. Bouanchaud, R. P. Novick, and Y. A. Chabbert.** 1980. Epidemiological and structural studies of *Staphylococcus aureus* R plasmids mediating resistance to tobramycin and streptogramin. Plasmid **4**:117–120.

126a. **El Solh, N., N. Moreau, and S. D. Ehrlich.** 1986. Molecular cloning and analysis of *Staphylococcus aureus* chromosomal aminoglycoside resistance genes. Plasmid **15**:104–118.

127. **El Solh, N., N. Moreau, B. Michel, and S. D. Ehrlich.** 1985. Analysis of the genes specifying aminoglycoside resistance in "methicillin-resistant" *Staphylococcus aureus* strains isolated in France. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl. **14**:575–576.
128. **Emslie, K. R., D. E. Townsend, S. Bolton, and W. B. Grubb.** 1985. Two distinct resistance determinants to nucleic acid-binding compounds in *Staphylococcus aureus*? FEMS Microbiol. Lett. **27**:61–64.
129. **Emslie, K. R., D. E. Townsend, and W. B. Grubb.** 1985. A resistance determinant to nucleic acid-binding compounds in methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **20**:139–145.
130. **Emslie, K. R., D. E. Townsend, and W. B. Grubb.** 1986. Isolation and characterization of a family of small plasmids encoding resistance to nucleic acid-binding compounds in *Staphylococcus aureus*. J. Med. Microbiol. **22**:9–15.
131. **Engel, H. W. B., N. Soedirman, J. A. Rost, W. J. van Leeuwen, and J. D. A. van Embden.** 1980. Transferability of macrolide, lincomycin, and streptogramin resistances between group A, B, and D streptococci, *Streptococcus pneumoniae*, and *Staphylococcus aureus*. J. Bacteriol. **142**:407–413.
132. **Ericson, C.** 1969. Resistance to acriflavine and cadmium, and changed phage reactions-markers of a new staphylococcal penicillinase plasmid. Acta Pathol. Microbiol. Scand. **76**:333.
133. **Espersen, F., P. B. Nielsen, K. Lund, B. Sylvest, and K. Jensen.**

1982. Hospital-acquired infections in a burns unit caused by an imported strain of *Staphylococcus aureus* with unusual multiresistance. J. Hyg. **88**:535–541.
134. **Falkow, S.** 1975. Infectious multiple drug resistance. Pion Limited, London.
135. **Farber, B. F., and R. C. Moellering, Jr.** 1983. Retrospective study of the toxicity of preparations of vancomycin from 1974 to 1981. Antimicrob. Agents Chemother. **23**:138–141.
136. **Faville, R. J., Jr., D. E. Zaske, E. L. Kaplan, K. Crossley, L. D. Sabath, and P. G. Quie.** 1978. *Staphylococcus aureus* endocarditis: combined therapy with vancomycin and rifampin. J. Am. Med. Assoc. **240**:1963–1965.
137. **Feitelson, J. S., and J. Lederberg.** 1980. Crude lysates of *Staphylococcus aureus* can transform *Bacillus subtilis*. J. Gen. Microbiol. **116**:545–547.
137a. **Ferretti, J. J., K. S. Gilmore, and P. Courvalin.** 1986. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. J. Bacteriol. **167**:631–638.
138. **Finegold, S. M., and D. W. Gaylor.** 1960. Enterocolitis due to phage type 54 staphylococci resistant to kanamycin, neomycin, paromomycin and chloramphenicol. N. Engl. J. Med. **263**:1110–1116.
139. **Fitton, J. E., and W. V. Shaw.** 1979. Comparison of chloramphenicol acetyltransferase variants in staphylococci. Biochem. J. **177**:575–582.
140. **Fitzgerald, G. F., and D. B. Clewell.** 1985. A conjugative transposon (Tn*919*) in *Streptococcus sanguis*. Infect. Immun. **47**:415–420.
141. **Forbes, B. A., and D. R. Schaberg.** 1983. Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance. J. Bacteriol. **153**:627–634.
142. **Foster, T. J.** 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. **47**:361–409.
143. **Fouace, J.** 1974. Transfer of resistance plasmids in *Staphylococcus aureus*. Ann. Microbiol. (Paris) **125A**:517–520.
144. **Franke, A. E., and D. B. Clewell.** 1981. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J. Bacteriol. **145**:494–502.
145. **Frère, J.-M., and B. Joris.** 1985. Penicillin-sensitive enzymes in peptidoglycan biosynthesis. Crit. Rev. Microbiol. **11**:299–396.
146. **Fujisawa, Y., and B. Weisblum.** 1981. A family of r-determinants in *Streptomyces* spp. that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **146**:621–631.
147. **Gale, E. F., E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring.** 1981. The molecular basis of antibiotic action, 2nd ed. Wiley Interscience, London.
148. **Garrod, L. P.** 1957. The erythromycin group of antibiotics. Br. Med. J. **2**:57–63.
149. **Gedney, J., and R. W. Lacey.** 1982. Properties of methicillin-resistant staphylococci now endemic in Australia. Med. J. Aust. **1**:448–450.
150. **Georgopapadakou, N. H., S. A. Smith, and D. P. Bonner.** 1982. Penicillin-binding proteins in a *Staphylococcus aureus* strain resistant to specific β-lactam antibiotics. Antimicrob. Agents Chemother. **22**:172–175.
151. **Giamarellou, H., M. Papapetropoulou, and G. K. Daikos.** 1981. Methicillin resistant *Staphylococcus aureus* infections during 1978–1979: clinical and bacteriologic observations. J. Antimicrob. Chemother. **7**:649–655.
152. **Gilbert, G. L., V. Asche, A. S. Hewstone, and J. L. Mathiesen.** 1982. Methicillin-resistant *Staphylococcus aureus* in neonatal nurseries. Two years' experience in special-care nurseries in Melbourne. Med. J. Aust. **1**:455–459.
153. **Gillespie, M. T., J. W. May, and R. A. Skurray.** 1984. Antibiotic susceptibilities and plasmid profiles of nosocomial methicillin-resistant *Staphylococcus aureus*: a retrospective study. J. Med. Microbiol. **17**:295–310.
154. **Gillespie, M. T., J. W. May, and R. A. Skurray.** 1985. Antibiotic resistance in *Staphylococcus aureus* isolated at an Australian hospital between 1946 and 1981. J. Med. Microbiol. **19**:137–147.
155. **Gillespie, M. T., J. W. May, and R. A. Skurray.** 1986. Plasmid-encoded resistance to acriflavine and quaternary ammonium compounds in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol. Lett. **34**:47–51.
156. **Gillespie, M. T., J. W. May, and R. A. Skurray.** 1986. Detection of an integrated tetracycline-resistance plasmid in the chromosome of methicillin resistant *Staphylococcus aureus*. J. Gen. Microbiol. **132**:1723–1728.
157. **Gillespie, M. T., and R. A. Skurray.** 1986. Plasmids in multiresistant *Staphylococcus aureus*. Microbiol. Sci. **3**:53–58.
158. **Goering, R. V., and D. K. Asch.** 1985. Plasmid mobilization effected by a conjugal aminoglycoside-resistance plasmid in *Staphylococcus aureus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl. **14**:563–565.
159. **Goering, R. V., and E. A. Ruff.** 1983. Comparative analysis of conjugative plasmids mediating gentamicin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **24**:450–452.
160. **Goering, R. V., B. A. Teeman, and E. A. Ruff.** 1985. Comparative physical and genetic maps of conjugal plasmids mediating aminoglycoside resistance in *Staphylococcus aureus* strains in the United States. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl. **14**:625–628.
161. **Goessens, W. H. F., P. Fontijne, M. van Raffe, and M. F. Michel.** 1984. Tolerance percentage as a criterion for the detection of tolerant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **25**:575–578.
162. **Gopal, V., A. L. Bisno, and F. J. Silverblatt.** 1976. Failure of vancomycin treatment in *Staphylococcus aureus* endocarditis. J. Am. Med. Assoc. **236**:1604–1606.
163. **Gorini, L.** 1974. Streptomycin and misreading of the genetic code, p. 791–803. *In* M. Nomura, A. Tissieres, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
164. **Götz, F., S. Ahrné, and M. Lindberg.** 1981. Plasmid transfer and genetic recombination by protoplast fusion in staphylococci. J. Bacteriol. **145**:74–81.
165. **Götz, F., J. Zabielski, L. Philipson, and M. Lindberg.** 1983. DNA homology between the arsenate resistance plasmid pSX267 from *Staphylococcus xylosus* and the penicillinase plasmid pI258 from *Staphylococcus aureus*. Plasmid **9**:126–137.
166. **Goulett, P. H., and H. S. Hieng.** 1981. Progression de la résistance à la gentamicine et à la tobramycine des souches de *Staphylococcus aureus* isolées d'infections hospitalières. Nouv. Presse Med. **10**:2645–2652.
167. **Goze, A., and S. D. Ehrlich.** 1980. Replication of plasmids from *Staphylococcus aureus* in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:7333–7337.
168. **Graham, M. Y., and B. Weisblum.** 1979. 23S ribosomal ribonucleic acid of macrolide-producing streptomycetes contains methylated adenine. J. Bacteriol. **137**:1464–1467.
169. **Gray, G. S.** 1983. Characterization of plasmids in aminocyclitol-resistant *Staphylococcus aureus*: electron microscopic and restriction endonuclease analysis. Plasmid **9**:159–181.
170. **Gray, G. S., and W. M. Fitch.** 1983. Evolution of antibiotic resistance genes: the DNA sequences of a kanamycin resistance gene from *Staphylococcus aureus*. Mol. Biol. Evol. **1**:57–66.
171. **Gray, G. S., R.T.-S. Huang, and J. Davies.** 1983. Aminocyclitol resistance in *Staphylococcus aureus*: presence of plasmids and aminocyclitol-modifying enzymes. Plasmid **9**:147–158.
172. **Grinsted, J., and R. W. Lacey.** 1973. Genetic variation of streptomycin resistance in clinical strains of *Staphylococcus aureus*. J. Med. Microbiol. **6**:351–361.
173. **Groves, D. J.** 1979. Interspecific relationships of antibiotic resistance in *Staphylococcus* sp: isolation and comparison of

plasmids determining tetracycline resistance in *S. aureus* and *S. epidermidis*. Can. J. Microbiol. **25**:1468–1475.
174. **Grubb, W. B., and D. I. Annear.** 1972. Spontaneous loss of methicillin resistance in *Staphylococcus aureus* at room temperature. Lancet **ii**:1257.
175. **Grubb, W. B., and D. I. Annear.** 1981. Unstable drug resistance in *Staphylococcus aureus* M4. Genet. Res. **38**:217–223.
176. **Grubb, W. B., and R. J. O'Reilly.** 1971. Joint transduction of separate extrachromosomal drug resistance determinants in *Staphylococcus aureus* E169. Biochem. Biophys. Res. Commun. **42**:377–383.
177. **Grubb, W. B., R. J. O'Reilly, and J. W. May.** 1972. Segregation of co-transduced streptomycin and tetracycline resistance in *Staphylococcus aureus*. Genet. Res. **20**:43–50.
178. **Gryczan, T. J., S. Contente, and D. Dubnau.** 1978. Characterization of *Staphylococcus aureus* plasmids introduced by transformation into *Bacillus subtilis*. J. Bacteriol. **134**:318–329.
179. **Gryczan, T. J., G. Grandi, J. Hahn, R. Grandi, and D. Dubnau.** 1980. Conformational alteration of mRNA structure and the posttranscriptional regulation of erythromycin-induced drug resistance. Nucleic Acids Res. **8**:6081–6097.
180. **Gryczan, T. J., J. Hahn, S. Contente, and D. Dubnau.** 1982. Replication and incompatibility properties of plasmid pE194 in *Bacillus subtilis*. J. Bacteriol. **152**:722–735.
181. **Gryczan, T., M. Israeli-Reches, M. Del Bue, and D. Dubnau.** 1984. DNA sequence and regulation of *ermD*, a macrolide-lincosamide-streptogramin B resistance element from *Bacillus licheniformis*. Mol. Gen. Genet. **194**:349–356.
182. **Guild, W. R., M. D. Smith, and N. B. Shoemaker.** 1982. Conjugative transfer of chromosomal R determinants in *Streptococcus pneumoniae*, p. 88–92. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
183. **Haley, R. W., and D. A. Bregman.** 1982. The role of understaffing and overcrowding in recurrent outbreaks of staphylococcal infection in a neonatal special-care unit. J. Infect. Dis. **145**:875–885.
184. **Haley, R. W., A. W. Hightower, R. F. Khabbaz, C. Thornsberry, W. J. Martone, J. R. Allen, and J. M. Hughes.** 1982. The emergence of methicillin-resistant *Staphylococcus aureus* infections in United States hospitals. Ann. Intern. Med. **97**:297–308.
185. **Hall, B. M.** 1970. Mercury resistance of *Staphylococcus aureus*. J. Hyg. **68**:121–129.
186. **Hancock, R. E. W.** 1981. Aminoglycoside uptake and mode of action—with special reference to streptomycin and gentamicin. II. Effects of aminoglycosides on cells. J. Antimicrob. Chemother. **8**:429–445.
187. **Harmon, S. A., J. N. Baldwin, W. C. Tien, and D. B. Critz.** 1966. Co-transduction of the genetic determinants of the synthesis of penicillinase and methionine in *Staphylococcus aureus*. Can. J. Microbiol. **12**:973–981.
188. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158**:513–516.
189. **Hartman, B. J., and A. Tomasz.** 1986. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29**:85–92.
190. **Hayes, M. V., N. A. C. Curtiss, A. W. Wyke, and J. B. Ward.** 1981. Decreased affinity of a penicillin-binding protein for β-lactam antibiotics in a clinical isolate of *Staphylococcus aureus* resistant to methicillin. FEMS Microbiol. Lett. **10**:119–122.
191. **Hofemeister, J., M. Israeli-Reches, and D. Dubnau.** 1983. Integration of plasmid pE194 at multiple sites on the *Bacillus subtilis* chromosome. Mol. Gen. Genet. **189**:58–68.
192. **Hone, R., M. Cafferkey, C. T. Keane, M. Harte-Barry, E. Moorhouse, R. Carroll, F. Martin, and R. Ruddy.** 1981. Bacteraemia in Dublin due to gentamicin resistant *Staphylococcus aureus*. J. Hosp. Infect. **2**:119–126.
193. **Horaud, T., C. Le Bouguenec, and K. Pepper.** 1985. Molecular genetics of resistance to macrolides, lincosamides and streptogramin B (MLS) in streptococci. J. Antimicrob. Chemother. **16**:(Suppl. A):111–135.
194. **Horinouchi, S., and B. Weisblum.** 1980. Posttranscriptional modification of mRNA conformation: mechanism that regulates erythromycin-induced resistance. Proc. Natl. Acad. Sci. USA **77**:7079–7083.
195. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **150**:804–814.
196. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. J. Bacteriol. **150**:815–825.
197. **Hoshino, T., T. Ikeda, K. Furukawa, and N. Tomizuka.** 1985. Genetic relationship between pUB110 and antibiotic-resistant plasmids obtained from thermophilic bacilli. Can. J. Microbiol. **31**:614–619.
198. **Hoshino, T., T. Ikeda, H. Narushima, and N. Tomizuka.** 1985. Isolation and characterization of antibiotic-resistance plasmids in thermophilic bacilli. Can. J. Microbiol. **31**:339–345.
199. **Hoshino, T., T. Ikeda, N. Tomizuka, and K. Furukawa.** 1985. Nucleotide sequence of the tetracycline resistance gene of pTHT15, a thermophilic *Bacillus* plasmid: comparison with staphylococcal Tc^R controls. Gene **37**:131–138.
200. **Hotta, K., H. Yamamoto, Y. Okami, and H. Umezawa.** 1981. Resistance mechanisms of kanamycin-neomycin- and streptomycin-producing streptomycetes to aminoglycoside drugs. J. Antibiot. **34**:1175–1182.
201. **Iida, S., J. Meyer, and W. Arber.** 1983. Prokaryotic IS elements, p. 159–221. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
202. **Imanaka, T., M. Fujii, and S. Aiba.** 1981. Isolation and characterization of antibiotic resistance plasmids from thermophilic bacilli and construction of deletion plasmids. J. Bacteriol. **146**:1091–1097.
203. **Imsande, J.** 1973. Repressor and antirepressor in the regulation of staphylococcal penicillinase synthesis. Genetics **75**:1–17.
204. **Imsande, J.** 1978. Genetic regulation of penicillinase synthesis in gram-positive bacteria. Microbiol. Rev. **42**:67–83.
205. **Imsande, J., and J. L. Lilleholm.** 1976. Characterization of mutations in the penicillinase operon of *Staphylococcus aureus*. Mol. Gen. Genet. **147**:23–27.
206. **Imsande, J., and J. L. Lilleholm.** 1977. Nature of the plasmid-linked penicillinase regulatory region in *Staphylococcus aureus*. Mol. Gen. Genet. **153**:153–157.
207. **Iordănescu, S.** 1975. Recombinant plasmid obtained from two different, compatible staphylococcal plasmids. J. Bacteriol. **124**:597–601.
208. **Iordănescu, S.** 1976. Three distinct plasmids originating in the same *Staphylococcus aureus* strain. Arch. Roum. Pathol. Exp. Microbiol. **35**:111–118.
209. **Iordănescu, S.** 1977. Relationships between cotransducible plasmids in *Staphylococcus aureus*. J. Bacteriol. **129**:71–75.
210. **Iordănescu, S., and M. Surdeanu.** 1976. Two restriction and modification systems in *Staphylococcus aureus* NCTC8325. J. Gen. Microbiol. **96**:277–281.
211. **Iordănescu, S., and M. Surdeanu.** 1980. New incompatibility groups for *Staphylococcus aureus* plasmids. Plasmid **4**:256–260.
212. **Iordănescu, S., M. Surdeanu, P. Della Latta, and R. Novick.** 1978. Incompatibility and molecular relationships between small staphylococcal plasmids carrying the same resistance marker. Plasmid **1**:468–479.
213. **Israeli-Reches, M., Y. Weinrauch, and D. Dubnau.** 1984. Evolutionary relationships of the *Bacillus licheniformis* macrolide-lincosamide-streptogramin B resistance elements. Mol. Gen. Genet. **194**:362–367.
214. **Jackson, M. P., J. Desena, J. Lednicky, B. McPherson, R. Haile, R. G. Garrison, and M. Rogolsky.** 1983. Isolation and characterization of a bacteriophage factor that confers competence for genetic transformation to an exfoliative toxin-producing strain of *Staphylococcus aureus*. Infect. Immun. **39**:939–947.
215. **Jacobs, S. I., A. T. Willis, G. B. Ludlam, and G. M. Goodburn.**

1963. Studies on newly recognized strains of *Staphylococcus aureus* associated with hospital infection. Lancet **i**:972–973.
216. **Jaffe, H. W., H. M. Sweeney, C. Nathan, R. A. Weinstein, S. A. Kabins, and S. Cohen.** 1980. Identity and interspecific transfer of gentamicin-resistance plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Infect. Dis. **141**:738–747.
217. **Jaffe, H. W., H. M. Sweeney, R. A. Weinstein, S. A. Kabins, C. Nathan, and S. Cohen.** 1982. Structural and phenotypic varieties of gentamicin resistance plasmids in hospital strains of *Staphylococcus aureus* and coagulase-negative staphylococci. Antimicrob. Agents Chemother. **21**:773–779.
218. **Jalanko, A., J. Palva, and H. Söderlund.** 1981. Restriction maps of plasmids pUB110 and pBD9. Gene **14**:325–328.
219. **Jánosi, L., and É. Bán.** 1982. Localization of genes coding for macrolide resistance on the penicillinase plasmid of isolates of an epidemic *Staphylococcus aureus*. Acta Microbiol. Acad. Sci. Hung. **29**:187–200.
220. **Johnston, L. H., and K. G. H. Dyke.** 1969. Ethidium bromide resistance, a new marker on the staphylococcal penicillinase plasmid. J. Bacteriol. **100**:1413–1414.
221. **Johnston, L. H., and K. G. H. Dyke.** 1974. Staphylococcal penicillinase plasmids: studies on the reversion of a temperature-sensitive replication mutant to temperature stability. J. Gen. Microbiol. **82**:309–317.
222. **Jones, I. G., and M. Midgley.** 1985. Expression of a plasmid borne ethidium resistance determinant from *Staphylococcus* in *Escherichia coli*: evidence for an efflux system. FEMS Microbiol. Lett. **28**:355–358.
223. **Kayser, F. H.** 1975. Methicillin-resistant staphylococci 1965–75. Lancet **ii**:650–652.
223a. **Kayser, F. H., B. Berger-Bächi, and W. D. Beck.** 1986. Genetics of multiply-resistant *Staphylococcus aureus*. J. Hosp. Infect. **7**(Suppl. A):19–27.
224. **Kayser, F. H., F. Homberger, and M. Devaud.** 1981. Aminocyclitol-modifying enzymes specified by chromosomal genes in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19**:766–772.
225. **Kayser, F. H., and T. M. Mak.** 1972. Methicillin-resistant staphylococci. Am. J. Med. Sci. **264**:197–205.
226. **Kayser, F. H., J. Wüst, and P. Corrodi.** 1972. Transduction and elimination of resistance determinants in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **2**:217–223.
227. **Kayser, F. H., J. Wüst, and P. Santanam.** 1976. Genetic and molecular characterisation of resistance determinants in methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **9**:137–148.
228. **Keggins, K. M., P. S. Lovett, and E. J. Duvall.** 1978. Molecular cloning of genetically active fragments of *Bacillus* DNA in *Bacillus subtilis* and properties of the vector plasmid pUB110. Proc. Natl. Acad. Sci. USA **75**:1423–1429.
229. **Khan, S. A., G. K. Adler, and R. P. Novick.** 1982. Functional origin of replication of pT181 plasmid DNA is contained within a 168-base-pair segment. Proc. Natl. Acad. Sci. USA **79**:4580–4584.
230. **Khan, S. A., S. M. Carleton, and R. P. Novick.** 1981. Replication of plasmid pT181 DNA *in vitro*: requirement for a plasmid-encoded product. Proc. Natl. Acad. Sci. USA **78**:4902–4906.
231. **Khan, S. A., and R. P. Novick.** 1980. Terminal nucleotide sequences of Tn*551*, a transposon specifying erythromycin resistance in *Staphylococcus aureus*: homology with Tn*3*. Plasmid **4**:148–154.
232. **Khan, S. A., and R. P. Novick.** 1983. Complete nucleotide sequence of pT181, a tetracycline-resistance plasmid from *Staphylococcus aureus*. Plasmid **10**:251–259.
233. **Kigbo, E. P., D. E. Townsend, N. Ashdown, and W. B. Grubb.** 1985. Transposition of penicillinase determinants in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol. Lett. **28**:39–43.
234. **King, K., L. Brady, M. Thompson, and J. L. Harkness.** 1982. Antibiotic-resistant staphylococci in a teaching hospital. Med. J. Aust. **2**:461–465.
235. **Kirby, W. M. M.** 1944. Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. Science **99**:452–453.
236. **Kirby, W. M. M.** 1984. Vancomycin therapy of severe staphylococcal infections. J. Antimicrob. Chemother. **14**(Suppl. D):73–78.
237. **Klastersky, J., J. Beumer, and D. Daneau.** 1971. Bacteriophage types and antibiotic susceptibility of *Staphylococcus aureus*. Appl. Microbiol. **22**:1000–1007.
238. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:341–404.
239. **Koch, A. L.** 1981. Evolution of antibiotic resistance gene function. Microbiol. Rev. **45**:355–378.
240. **Koepsel, R. R., R. W. Murray, W. D. Rosenblum, and S. A. Khan.** 1985. The replication initiator protein of plasmid pT181 has sequence-specific endonuclease and topoisomerase-like activities. Proc. Natl. Acad. Sci. USA **82**:6845–6849.
241. **Kondo, I., T. Ishikawa, and H. Nakahara.** 1974. Mercury and cadmium resistances mediated by the penicillinase plasmid in *Staphylococcus aureus*. J. Bacteriol. **117**:1–7.
242. **Konno, M., K. Ubukata, H. Takahashi, Y. Sasaki, and S. Kawakami.** 1982. Gentamicin resistance in *Staphylococcus aureus* isolated in Japan. Part I. Isolation frequency of *Staphylococcus aureus* resistant to gentamicin and correlation of antibiotic susceptibilities with bacteriophage type. Chemotherapy (Tokyo) **30**:86–95.
243. **Kono, M., and M. Sasatsu.** 1976. Association of a penicillin resistance gene with a tetracycline resistance plasmid ($P_{TP\text{-}2}$) in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **9**:706–712.
244. **Kono, M., M. Sasatsu, K. Ubukata, M. Konno, and R. Fujii.** 1978. New plasmid (pTU512), mediating resistance to penicillin, erythromycin, and kanamycin, from clinical isolates of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **13**:691–694.
245. **Kozarich, J. W., and J. L. Strominger.** 1978. A membrane enzyme from *Staphylococcus aureus* which catalyzes transpeptidase, carboxypeptidase and penicillinase activities. J. Biol. Chem. **255**:1272–1278.
246. **Krogstad, D. J., R. M. Smith, R. C. Moellering, Jr., and A. R. Parquette.** 1980. Visualization of cell-cell contact during conjugation in *Streptococcus faecalis*. J. Bacteriol. **141**:963–967.
247. **Krolewski, J. J., E. Murphy, R. P. Novick, and M. G. Rush.** 1981. Site-specificity of the chromosomal insertion of *Staphylococcus aureus* transposon Tn*554*. J. Mol. Biol. **152**:19–33.
248. **Kuck, N. A., and M. Forbes.** 1973. Uptake of minocycline and tetracycline by tetracycline-susceptible and -resistant bacteria. Antimicrob. Agents Chemother. **3**:662–664.
249. **Kuhl, S. A., P. A. Pattee, and J. N. Baldwin.** 1978. Chromosomal map location of the methicillin resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **135**:460–465.
250. **Kumar, C. C., and R. P. Novick.** 1985. Plasmid pT181 replication is regulated by two countertranscripts. Proc. Natl. Acad. Sci. USA **82**:638–642.
251. **Lacey, R. W.** 1971. Transfer of tetracycline-resistance between strains of *Staphylococcus aureus* in mixed culture. J. Gen. Microbiol. **69**:229–237.
252. **Lacey, R. W.** 1971. High-frequency transfer of neomycin resistance between naturally occurring strains of *Staphylococcus aureus*. J. Med. Microbiol. **4**:73–84.
253. **Lacey, R. W.** 1972. Genetic control in methicillin-resistant strains of *Staphylococcus aureus*. J. Med. Microbiol. **5**:497–508.
254. **Lacey, R. W.** 1972. Transfer of chromosomal genes between staphylococci in mixed cultures. J. Gen. Microbiol. **71**:399–401.
255. **Lacey, R. W.** 1975. Antibiotic resistance plasmids of *Staphylococcus aureus* and their clinical importance. Bacteriol. Rev. **39**:1–32.
256. **Lacey, R. W.** 1980. Evidence for two mechanisms of plasmid transfer in mixed cultures of *Staphylococcus aureus in vitro*. J. Gen. Microbiol. **119**:423–435.
257. **Lacey, R. W.** 1980. Rarity of gene transfer between animal and human isolates of *Staphylococcus aureus*. J. Gen. Microbiol.

119:437–442.
258. **Lacey, R. W.** 1982. Do sulphonamide-trimethoprim combinations select less resistance to trimethoprim than the use of trimethoprim alone? J. Med. Microbiol. **15**:403–427.
259. **Lacey, R. W.** 1984. Antibiotic resistance in *Staphylococcus aureus* and streptococci. Br. Med. Bull. **40**:77–83.
260. **Lacey, R. W., and I. Chopra.** 1972. Evidence for mutation to streptomycin resistance in clinical strains of *Staphylococcus aureus*. J. Gen. Microbiol. **73**:175–180.
261. **Lacey, R. W., and I. Chopra.** 1974. Genetic studies of a multi-resistant strain of *Staphylococcus aureus*. J. Med. Microbiol. **7**:285–297.
262. **Lacey, R. W., and J. Grinsted.** 1972. Linkage of fusidic acid resistance to the penicillinase plasmid in *Staphylococcus aureus*. J. Gen. Microbiol. **73**:501–508.
263. **Lacey, R. W., and J. Grinsted.** 1973. Genetic analysis of methicillin-resistant strains of *Staphylococcus aureus*; evidence for their evolution from a single clone. J. Med. Microbiol. **6**:511–526.
264. **Lacey, R. W., N. Keyworth, and C. Lincoln.** 1984. Staphylococci in the U.K.: a review. J. Antimicrob. Chemother. **14**(Suppl. D):19–25.
265. **Lacey, R. W., E. Lewis, and V. T. Rosdahl.** 1974. Evolution of plasmids *in vivo* in a strain of *Staphylococcus aureus*. J. Med. Microbiol. **7**:117–125.
266. **Lacey, R. W., and V. L. Lord.** 1980. Transfer of gentamicin resistance between cultures of *Staphylococcus aureus* in nutrient broth, serum and urine. J. Med. Microbiol. **13**:411–421.
267. **Lacey, R. W., and V. L. Lord.** 1981. Sensitivity of staphylococci to fatty acids: novel inactivation of linolenic acid by serum. J. Med. Microbiol. **14**:41–49.
268. **Lacey, R. W., and A. A. B. Mitchell.** 1969. Gentamicin-resistant *Staphylococcus aureus*. Lancet **ii**:1425–1426.
269. **Lacey, R. W., and M. H. Richmond.** 1974. The genetic basis of antibiotic resistance in *S. aureus*: the importance of gene transfer in the evolution of this organism in the hospital environment. Ann. N.Y. Acad. Sci. **236**:395–412.
270. **Lacey, R. W., and V. T. Rosdahl.** 1974. An unusual "penicillinase plasmid" in *Staphylococcus aureus*; evidence for its transfer under natural conditions. J. Med. Microbiol. **7**:1–9.
271. **Lacey, R. W., and A. Stokes.** 1979. Studies on recently isolated cultures of methicillin-resistant *Staphylococcus aureus*. J. Gen. Microbiol. **114**:329–339.
272. **Lai, C. J., J. E. Dahlberg, and B. Weisblum.** 1973. Structure of an inducibly methylatable nucleotide sequence in 23S ribosomal ribonucleic acid from erythromycin-resistant *Staphylococcus aureus*. Biochemistry **12**:457–460.
273. **Lai, C. J., and B. Weisblum.** 1971. Altered methylation of ribosomal RNA in an erythromycin-resistant strain of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **68**:856–860.
274. **Lambert, H. P.** 1984. Impact of bacterial resistance to antibiotics on therapy. Br. Med. Bull. **40**:102–106.
275. **Lampson, B. C., and J. T. Parisi.** 1986. Naturally occurring *Staphylococcus epidermidis* plasmid expressing constitutive macrolide-lincosamide-streptogramin B resistance contains a deleted attenuator. J. Bacteriol. **166**:479–483.
276. **Landy, M., N. W. Larkum, E. J. Oswald, and P. Streighoff.** 1943. Increased synthesis of *p*-amino-benzoic acid associated with the development of sulfonamide resistance in *Staphylococcus aureus*. Science **97**:265–267.
277. **Le Goffic, F., M.-L. Capmau, J. Abbe, C. Cerceau, A. Dublanchet, and J. Duval.** 1977. Plasmid mediated pristinamycin resistance: PH 1A, a pristinamycin 1A hydrolase. Ann. Microbiol. (Paris) **128B**:471–474.
278. **Le Goffic, F., M. L. Capmau, D. Bonnet, C. Cerceau, C. Soussy, A. Dublanchet, and J. Duval.** 1977. Plasmid-mediated pristinamycin resistance. PAC IIA. A new enzyme which modifies pristinamycin IIA. J. Antibiot. **30**:665–669.
279. **Le Goffic, F., A. Martel, M.-L. Capmau, B. Baca, P. Geobel, H. Chardon, C. J. Soussy, J. Duval, and D. H. Bouanchaud.** 1976. New plasmid-mediated nucleotidylation of aminoglycoside antibiotics in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **10**:258–264.
280. **Le Goffic, F., A. Martel, N. Moreau, M.-L. Capmau, C. J. Soussy, and J. Duval.** 1977. 2″-*O*-Phosphorylation of gentamicin components by a *Staphylococcus aureus* strain carrying a plasmid. Antimicrob. Agents Chemother. **12**:26–30.
281. **Le Goffic, F., N. Moreau, and M. Masson.** 1977. Are some aminoglycoside antibiotics inactivating enzymes polyfunctional? Ann. Microbiol. (Paris) **128B**:465–469.
282. **Levy, S. B.** 1982. Microbial resistance to antibiotics: an evolving and persistent problem. Lancet **ii**:83–88.
283. **Levy, S. B.** 1984. Resistance to the tetracyclines, p. 191–240. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
284. **Lewis, E. L., and R. W. Lacey.** 1973. Present significance of resistance to trimethoprim and sulfonamides in coliforms, *Staphylococcus aureus* and *Streptococcus faecalis*. J. Clin. Pathol. **26**:175–180.
285. **Lindberg, M., and R. P. Novick.** 1973. Plasmid-specific transformation in *Staphylococcus aureus*. J. Bacteriol. **115**:139–145.
286. **Lindberg, M., J.-E. Sjöström, and T. Johansson.** 1972. Transformation of chromosomal and plasmid characters in *Staphylococcus aureus*. J. Bacteriol. **109**:844–847.
287. **Löfdahl, S., J.-E. Sjöström, and L. Philipson.** 1978. Characterization of small plasmids from *Staphylococcus aureus*. Gene **3**:149–159.
288. **Löfdahl, S., J.-E. Sjöström, and L. Philipson.** 1978. A vector for recombinant DNA in *Staphylococcus aureus*. Gene **3**:161–172.
289. **Löfdahl, S., J. Zabielski, and L. Philipson.** 1981. Structure and restriction enzyme maps of the circularly permuted DNA of staphylococcal bacteriophage ϕ11. J. Virol. **37**:784–794.
290. **Lowbury, E. J. L., J. R. Babb, V. I. Brown, and B. J. Collins.** 1964. Neomycin-resistant *Staphylococcus aureus* in a burns unit. J. Hyg. **62**:221–228.
291. **Lowbury, E. J. L., D. M. Jackson, C. R. Ricketts, and B. Davis.** 1971. Topical chemoprophylaxis for burns: trials of creams containing silver sulphadiazine and trimethoprim. Injury **3**:18–24.
292. **Luchansky, J. B., and P. A. Pattee.** 1984. Isolation of transposon Tn*551* insertions near chromosomal markers of interest in *Staphylococcus aureus*. J. Bacteriol. **159**:894–899.
292a. **Lyon, B. R., M. T. Gillespie, M. E. Byrne, J. W. May, and R. A. Skurray.** 1987. Plasmid-mediated resistance to gentamicin in *S. aureus*: the involvement of a transposon. J. Med. Microbiol. **23**:105–114.
293. **Lyon, B. R., J. L. Iuorio, J. W. May, and R. A. Skurray.** 1984. Molecular epidemiology of multiresistant *Staphylococcus aureus* in Australian hospitals. J. Med. Microbiol. **17**:79–89.
294. **Lyon, B. R., J. W. May, J. H. Marshall, and R. A. Skurray.** 1982. Plasmid-mediated antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. Med. J. Aust. **1**:468–469.
295. **Lyon, B. R., J. W. May, and R. A. Skurray.** 1983. Analysis of plasmids in nosocomial strains of multiple-antibiotic-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **23**:817–826.
296. **Lyon, B. R., J. W. May, and R. A. Skurray.** 1984. Tn*4001*: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. Mol. Gen. Genet. **193**:554–556.
297. **Lyon, B. R., J. M. Tennent, J. W. May, and R. A. Skurray.** 1986. Trimethoprim resistance encoded on a *S. aureus* gentamicin resistance plasmid: cloning and transposon mutagenesis. FEMS Microbiol. Lett. **33**:189–192.
298. **Mandel, L. J., E. Murphy, N. H. Steigbigel, and M. H. Miller.** 1984. Gentamicin uptake in *Staphylococcus aureus* possessing plasmid-encoded, aminoglycoside-modifying enzymes. Antimicrob. Agents Chemother. **26**:563–569.
299. **Mandell, G. L., and D. R. Moorman.** 1980. Treatment of experimental staphylococcal infections: effect of rifampin alone and in combination on development of rifampin resistance. Antimicrob. Agents Chemother. **17**:658–662.
300. **Martel, A., M. Masson, N. Moreau, and F. Le Goffic.** 1983. Kinetic studies of aminoglycoside acetyltransferase and phosphotransferase from *Staphylococcus aureus* RPAL. Relationship between the two activities. Eur. J. Biochem. **133**:515–521.

301. **Mates, S. M., E. S. Eisenberg, L. J. Mandel, L. Patel, H. R. Kaback, and M. H. Miller.** 1982. Membrane potential and gentamicin uptake in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **79**:6693–6697.
302. **Mates, S. M., L. Patel, H. R. Kaback, and M. H. Miller.** 1983. Membrane potential in anaerobically growing *Staphylococcus aureus* and its relationship to gentamicin uptake. Antimicrob. Agents Chemother. **23**:526–530.
302a. **Matsuhashi, M., M. D. Song, F. Ishimo, M. Wachi, M. Doi, M. Inoue, K. Ubukata, N. Yamashita, and M. Konno.** 1986. Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to β-lactam antibiotics in *Staphylococcus aureus*. J. Bacteriol. **167**:975–980.
303. **Matsumura, M., Y. Katakura, T. Imanaka, and S. Aiba.** 1984. Enzymatic and nucleotide sequence studies of a kanamycin-inactivating enzyme encoded by a plasmid from thermophilic bacilli in comparison with that encoded by plasmid pUB110. J. Bacteriol. **160**:413–420.
304. **Matthews, P. R., and P. R. Stewart.** 1984. Resistance heterogeneity in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol. Lett. **22**:161–166.
305. **Mayford, M., and B. Weisblum.** 1985. Messenger RNA from *Staphylococcus aureus* that specifies macrolide-lincosamide-streptogramin resistance: demonstration of its conformations and of the leader peptide it encodes. J. Mol. Biol. **185**:769–780.
306. **Mayhall, C. G., G. Medoff, and J. J. Marr.** 1976. Variation in the susceptibility of strains of *Staphylococcus aureus* to oxacillin, cephalothin, and gentamicin. Antimicrob. Agents Chemother. **10**:707–712.
307. **McDonald, M. I., I. Graham, K. J. Harvey, and A. Sinclair.** 1981. Antibacterial activity of hydrolysed linseed oil and linolenic acid against methicillin-resistant *Staphylococcus aureus*. Lancet **ii**:1056.
308. **McDonnell, R. W., H. M. Sweeney, and S. Cohen.** 1983. Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **23**:151–160.
309. **McGowan, J. E., Jr.** 1983. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. Rev. Infect. Dis. **5**:1033–1048.
310. **McGowan, J. E., Jr., P. M. Terry, T.-S. R. Huang, C. L. Houk, and J. Davies.** 1979. Nosocomial infections with gentamicin-resistant *Staphylococcus aureus*: plasmid analysis as an epidemiologic tool. J. Infect. Dis. **140**:864–872.
311. **McKenzie, T., T. Hoshino, T. Tanaka, and N. Sueoka.** 1986. The nucleotide sequence of pUB110: some salient features in relation to replication and its regulation. Plasmid **15**:93–104.
312. **McLaughlin, J. R., C. L. Murray, and J. C. Rabinowitz.** 1981. Plasmid-directed expression of *Staphylococcus aureus* β-lactamase by *Bacillus subtilis in vitro*. J. Biol. Chem. **256**:11273–11282.
313. **McLaughlin, J. R., C. R. Murray, and J. C. Rabinowitz.** 1981. Unique features in the ribosome binding site sequence of the Gram-positive *Staphylococcus aureus* β-lactamase gene. J. Biol. Chem. **256**:11283–11291.
314. **McMurry, L. M., J. C. Cullinane, R. E. Petrucci, Jr., and S. B. Levy.** 1981. Active uptake of tetracycline by membrane vesicles from susceptible *Escherichia coli*. Antimicrob. Agents Chemother. **20**:307–313.
315. **McMurry, L. M., R. E. Petrucci, Jr., and S. B. Levy.** 1980. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:3974–3977.
316. **Medeiros, A. A.** 1984. β-Lactamases. Br. Med. Bull. **40**:18–27.
317. **Meijers, J. A., and E. E. Stobberingh.** 1980. Chromosomal penicillin resistance in *Staphylococcus aureus* strains of phage group II. Antonie van Leeuwenhoek J. Microbiol. Serol. **46**:577–586.
318. **Meijers, J. A., K. C. Winkler, and E. E. Stobberingh.** 1981. Resistance transfer in mixed cultures of *Staphylococcus aureus*. J. Med. Microbiol. **14**:21–39.
319. **Meyers, J. A., E. E. Stobberingh, and K. C. Winkler.** 1979. Restriction of resistance transfer in *Staphylococcus aureus*. Contrib. Microbiol. Immunol. **6**:189–197.
320. **Mickelsen, P. A., J. J. Plorde, K. P. Gordon, C. Hargiss, J. McClure, F. D. Schoenknecht, F. Condie, F. C. Tenover, and L. S. Tompkins.** 1985. Instability of antibiotic resistance in a strain of *Staphylococcus epidermidis* isolated from an outbreak of prosthetic valve endocarditis. J. Infect. Dis. **152**:50–58.
321. **Miller, M. H., S. C. Edberg, L. J. Mandel, C. F. Behar, and N. H. Steigbigel.** 1980. Gentamicin uptake in wild-type and aminoglycoside-resistant small-colony mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **18**:722–729.
322. **Mitsuhashi, S., H. Hashimoto, M. Kono, and M. Morimura.** 1965. Drug resistance of staphylococci. II. Joint elimination and joint transduction of the determinants of penicillinase production and resistance to macrolide antibiotics. J. Bacteriol. **89**:988–992.
323. **Moorman, D. R., and G. L. Mandell.** 1981. Characteristics of rifampin-resistant variants obtained from clinical isolates of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **20**:709–713.
324. **Morimura, M., K. Watanabe, E. Mori, and S. Mitsuhashi.** 1970. Lability of streptomycin resistance in *Staphylococcus aureus*. Jpn. J. Microbiol. **14**:253–256.
325. **Morrow, T. O., and S. A. Harmon.** 1979. Genetic analysis of *Staphylococcus aureus* RNA polymerase mutants. J. Bacteriol. **137**:374–383.
326. **Muller, R. E., T. Ano, T. Imanaka, and S. Aiba.** 1986. Complete nucleotide sequences of *Bacillus* plasmids pUB110dB, pRBH1 and its copy mutants. Mol. Gen. Genet. **202**:169–171.
327. **Murphy, E.** 1983. Inhibition of Tn*554* transposition: deletion analysis. Plasmid **10**:260–269.
328. **Murphy, E.** 1985. Nucleotide sequence of *ermA*, a macrolide-lincosamide-streptogramin B determinant in *Staphylococcus aureus*. J. Bacteriol. **162**:633–640.
329. **Murphy, E.** 1985. Nucleotide sequence of a spectinomycin adenyltransferase AAD(9) determinant from *Staphylococcus aureus* and its relationship to AAD(3")(9). Mol. Gen. Genet. **200**:33–39.
330. **Murphy, E., L. Huwyler, and M. C. F. Bastos.** 1985. Transposon Tn*554*: complete nucleotide sequence and isolation of transposition-defective and antibiotic-sensitive mutants. EMBO J. **4**:3357–3365.
331. **Murphy, E., and S. Löfdahl.** 1984. Transposition of Tn*554* does not generate a target duplication. Nature (London) **307**:292–294.
332. **Murphy, E., and R. P. Novick.** 1979. Physical mapping of *Staphylococcus aureus* penicillinase plasmid pI524: characterization of an invertible region. Mol. Gen. Genet. **175**:19–30.
333. **Murphy, E., and R. P. Novick.** 1980. Site-specific recombination between plasmids of *Staphylococcus aureus*. J. Bacteriol. **141**:316–326.
334. **Murphy, E., S. Phillips, I. Edelman, and R. P. Novick.** 1981. Tn*554*: isolation and characterization of plasmid insertions. Plasmid **5**:292–305.
335. **Naidoo, J.** 1984. Interspecific co-transfer of antibiotic resistance plasmids in staphylococci *in vivo*. J. Hyg. **93**:59–66.
336. **Naidoo, J., and D. H. Lloyd.** 1984. Transmission of genes between staphylococci on skin, p. 285–292. *In* M. Woodbine (ed.), Antimicrobials and agriculture. Butterworths, London.
337. **Naidoo, J., and W. C. Noble.** 1978. Transfer of gentamicin resistance between strains of *Staphylococcus aureus* on skin. J. Gen. Microbiol. **107**:391–393.
338. **Naidoo, J., and W. C. Noble.** 1981. Transfer of gentamicin resistance between coagulase-negative and coagulase-positive staphylococci on skin. J. Hyg. **86**:183–187.
339. **Naidoo, J., W. C. Noble, A. Weissmann, and K. G. H. Dyke.** 1983. Gentamicin-resistant staphylococci: genetics of an outbreak in a dermatology department. J. Hyg. **91**:7–16.
340. **Narayanan, C. S., and D. Dubnau.** 1985. Evidence for the translational attenuation model: ribosome-binding studies and structural analysis with an *in vitro* run-off transcript of *ermC*. Nucleic Acids Res. **13**:7307–7326.

341. **Noble, W. C., and J. Naidoo.** 1978. Evolution of antibiotic resistance in *Staphylococcus aureus*: the role of the skin. Br. J. Dermatol. **98**:481–489.
342. **Noguchi, N., K. Shishido, T. Ando, and M. Kono.** 1983. Construction and propagation of deletion derivatives of staphylococcal tetracycline-resistance plasmid pTP-5 in *Bacillus subtilis*. Gene **21**:105–110.
343. **North, E. A., and Christie, R.** 1946. Acquired resistance of staphylococci to the action of penicillin. Med. J. Aust. **1**:176–179.
344. **Novick, R. P.** 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. J. Gen. Microbiol. **33**:121–136.
345. **Novick, R.** 1967. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. Virology **33**:155–166.
346. **Novick, R.** 1976. Plasmid-protein relaxation complexes in *Staphylococcus aureus*. J. Bacteriol. **127**:1177–1187.
347. **Novick, R. P., G. K. Adler, S. Majumder, S. A. Khan, S. Carleton, W. D. Rosenblum, and S. Iordanescu.** 1982. Coding sequence for the pT181 *repC* product: a plasmid-coded protein uniquely required for replication. Proc. Natl. Acad. Sci. USA **79**:4108–4112.
348. **Novick, R. P., and D. Bouanchaud.** 1971. Extrachromosomal nature of drug resistance in *S. aureus*. Ann. N.Y. Acad. Sci. **182**:279–294.
349. **Novick, R. P., I. Edelman, P. Della Latta, E. C. Swanson, and P. A. Pattee.** 1979. Translocatable elements in *Staphylococcus aureus*. Contrib. Microbiol. Immunol. **6**:41–55.
349a. **Novick, R. P., I. Edelman, and S. Lofdahl.** 1986. Small *Staphylococcus aureus* plasmids are transduced as linear multimers that are formed and resolved by replication processes. J. Mol. Biol. **192**:209–220.
350. **Novick, R. P., I. Edelman, M. D. Schwesinger, A. D. Gruss, E. C. Swanson, and P.A. Pattee.** 1979. Genetic translocation in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **76**:400–404.
351. **Novick, R. P., S. Iordănescu, M. Surdeanu, and I. Edelman.** 1981. Transduction-related cointegrate formation between staphylococcal plasmids: a new type of site-specific recombination. Plasmid **6**:159–172.
352. **Novick, R. P., S. A. Khan, E. Murphy, S. Iordănescu, I. Edelman, J. Krolewski, and M. Rush.** 1981. Hitchhiking transposons and other mobile genetic elements and site-specific recombination systems in *Staphylococcus aureus*. Cold Spring Harbor Symp. Quant. Biol. **45**:67–76.
353. **Novick, R. P., and S. I. Morse.** 1967. *In vivo* transmission of drug resistance factors between strains of *Staphylococcus aureus*. J. Exp. Med. **125**:45–59.
354. **Novick, R. P., and E. Murphy.** 1985. MLS-resistance determinants in *Staphylococcus aureus* and their molecular evolution. J. Antimicrob. Chemother. **16**(Suppl. A):101–110.
355. **Novick, R. P., E. Murphy, T. J. Gryczan, E. Baron, and I. Edelman.** 1979. Penicillinase plasmids of *Staphylococcus aureus*: restriction-deletion maps. Plasmid **2**:109–129.
356. **Novick, R. P., S. J. Projan, C. C. Kumar, S. Carleton, A. Gruss, S. K. Highlander, and J. Kornblum.** 1985. Replication control for pT181, an indirectly regulated plasmid, p. 299–320. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
357. **Novick, R., S. J. Projan, W. Rosenblum, and I. Edelman.** 1984. Staphylococcal plasmid cointegrates are formed by host- and phage-mediated general *rec* systems that act on short regions of homology. Mol. Gen. Genet. **195**:374–377.
358. **Novick, R. P., and C. Roth.** 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J. Bacteriol. **95**:1335–1342.
359. **Novick, R. P., C. Sanchez-Rivas, A. Gruss, and I. Edelman.** 1980. Involvement of the cell envelope in plasmid maintenance: plasmid curing during the regeneration of protoplasts. Plasmid **3**:348–358.
360. **Oeding, P.** 1983. Taxonomy and identification, p. 1–31. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 1. Clinical and epidemiological aspects. Academic Press, Inc. (London), Ltd., London.
361. **Ohtsubo, H., H. Ohmori, and E. Ohtsubo.** 1979. Nucleotide sequence analysis of Tn*3* (Ap): implications for insertion and deletion. Cold Spring Harbor Symp. Quant. Biol. **45**:1269–1278.
362. **O'Toole, R. D., W. L. Drew, B. J. Dahlgren, and H. N. Beaty.** 1970. An outbreak of methicillin-resistant *Staphylococcus aureus* infection: observations in hospital and nursing home. J. Am. Med. Assoc. **213**:257–263.
363. **Parisi, J. T.** 1985. Coagulase-negative staphylococci and the epidemiological typing of *Staphylococcus epidermidis*. Microbiol. Rev. **49**:126–139.
364. **Parisi, J. T., J. Robbins, B. C. Lampson, and D. W. Hecht.** 1981. Characterization of a macrolide, lincosamide, and streptogramin resistance plasmid in *Staphylococcus epidermidis*. J. Bacteriol. **148**:559–564.
365. **Pariza, M. W., and J. J. Iandolo.** 1974. Base ratio and deoxyribonucleic acid homology studies of six *Staphylococcus aureus* typing bacteriophages. Appl. Microbiol. **27**:317–323.
366. **Pariza, M. W., and J. J. Iandolo.** 1974. Determination of genome size of selected typing bacteriophages of *Staphylococcus aureus*. Appl. Microbiol. **28**:510–512.
367. **Parker, M. T.** 1983. The significance of phage-typing patterns in *Staphylococcus aureus*, p. 33–62. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 1. Clinical and epidemiological aspects. Academic Press, Inc. (London), Ltd., London.
368. **Parker, M. T., E. H. Asheshov, J. H. Hewitt, L. S. Nakhla, and B. M. Brock.** 1974. Endemic staphylococcal infections in hospitals. Ann. N.Y. Acad. Sci. **236**:466–484.
369. **Parker, M. T., and J. H. Hewitt.** 1970. Methicillin resistance in *Staphylococcus aureus*. Lancet **i**:800–804.
370. **Parr, T. R., Jr., and L. E. Bryan.** 1984. Nonenzymatic resistance to β-lactam antibiotics and resistance to other cell wall synthesis inhibitors, p. 81–111. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
371. **Pattee, P. A.** 1976. Genetic linkage of chromosomal tetracycline resistance and pigmentation to a purine auxotrophic marker and the isoleucine-valine-leucine structural genes in *Staphylococcus aureus*. J. Bacteriol. **127**:1167–1172.
372. **Pattee, P. A.** 1981. Distribution of Tn*551* insertion sites responsible for auxotrophy on the *Staphylococcus aureus* chromosome. J. Bacteriol. **145**:479–488.
373. **Pattee, P. A., J. M. Jones, and S. C. Yost.** 1984. Chromosome map of *Staphylococcus aureus*, p. 126–130. *In* S. J. O'Brien (ed.), Genetic maps 1984: a compilation of linkage and restriction maps of genetically studied organisms, vol. 3. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
374. **Pattee, P. A., and D. S. Neveln.** 1975. Transformation analysis of three linkage groups in *Staphylococcus aureus*. J. Bacteriol. **124**:201–211.
375. **Pattee, P. A., N. E. Thompson, D. Haubrich, and R. P. Novick.** 1977. Chromosomal map locations of integrated plasmids and related elements in *Staphylococcus aureus*. Plasmid **1**:38–51.
376. **Pavillard, R., K. Harvey, D. Douglas, A. Hewstone, J. Andrew, B. Collopy, V. Asche, P. Carson, A. Davidson, G. Gilbert, J. Spicer, and F. Tosolini.** 1982. Epidemic of hospital-acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victorian hospitals. Med. J. Aust. **1**:451–454.
377. **Pearman, J. W., K. J. Christiansen, D. I. Annear, C. S. Goodwin, C. Metcalf, F. P. Donovan, K. L. Macey, L. D. Bassette, I. M. Powell, J. M. Green, W. E. Harper, and M. S. McKelvie.** 1985. Control of methicillin-resistant *Staphylococcus aureus* (MRSA) in an Australian metropolitan teaching hospital complex. Med. J. Aust. **142**:103–108.
378. **Perceval, A., A. J. McLean, and C. V. Wellington.** 1976. Emergence of gentamicin resistance in *Staphylococcus aureus*. Med. J. Aust. **2**:74.
379. **Perkins, J. B., and P. Youngman.** 1983. *Streptococcus* plasmid pAMα1 is a composite of two separable replicons, one of

which is closely related to *Bacillus* plasmid pBC16. J. Bacteriol. **155**:607–615.
380. **Perkins, J. B., and P. J. Youngman.** 1984. A physical and functional analysis of Tn*917*, a *Streptococcus* transposon in the Tn*3* family that functions in *Bacillus*. Plasmid **12**:119–138.
381. **Perry, R. D., and S. Silver.** 1982. Cadmium and manganese transport in *Staphylococcus aureus* membrane vesicles. J. Bacteriol. **150**:973–976.
382. **Peyru, G., L. F. Wexler, and R. P. Novick.** 1969. Naturally occurring penicillinase plasmids in *Staphylococcus aureus*. J. Bacteriol. **98**:215–221.
383. **Phillips, I., and K. Shannon.** 1984. Aminoglycoside resistance. Br. Med. Bull. **40**:28–35.
384. **Phillips, S., and R. P. Novick.** 1979. Tn*554* - a site-specific repressor controlled transposon in *Staphylococcus aureus*. Nature (London) **278**:476–478.
385. **Pittman, M. A., H. H. Stone, and L. Kolb.** 1965. Use of gentamicin sulfate for preoperative bowel sterilization, p. 160–163. Antimicrob. Agents Chemother. 1964.
386. **Plorde, J. J., and J. C. Sherris.** 1974. Staphylococcal resistance to antibiotics: origin, measurement, and epidemiology. Ann. N.Y. Acad. Sci. **236**:413–434.
387. **Polak, J., and R. Novick.** 1982. Closely related plasmids from *Staphylococcus aureus* and soil bacilli. Plasmid **7**:152–162.
388. **Pollock, M. R.** 1967. Origin and function of penicillinase: a problem in biochemical evolution. Br. Med. J. **4**:71–77.
389. **Porter, F. D., S. Silver, C. Ong, and H. Nakahara.** 1982. Selection for mercurial resistance in hospital settings. Antimicrob. Agents Chemother. **22**:852–858.
390. **Porthouse, A., D. F. J. Brown, R. G. Smith, and T. Rogers.** 1976. Gentamicin resistance in *Staphylococcus aureus*. Lancet **i**:20–21.
391. **Poston, S. M.** 1966. Cellular location of the genes controlling penicillinase production and resistance to streptomycin and tetracycline in a strain of *Staphylococcus aureus*. Nature (London) **210**:802–804.
392. **Poston, S. M., and J. L. Naidoo.** 1983. Genetics and antimicrobial drug resistance in the staphylococci, p. 63–119. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 1. Clinical and epidemiological aspects. Academic Press, Inc. (London), Ltd., London.
393. **Price, E. H., A. Brain, and J. A. S. Dickson.** 1980. An outbreak of infection with a gentamicin and methicillin resistant *Staphylococcus aureus* in a neonatal unit. J. Hosp. Infect. **1**:221–228.
394. **Projan, S. J., J. Kornblum, S. L. Moghazeh, I. Edelman, M. L. Gennaro, and R. P. Novick.** 1985. Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *Staphylococcus aureus*. Mol. Gen. Genet. **199**:452–464.
395. **Qoronfleh, M. W., and B. J. Wilkinson.** 1986. Effects of growth of methicillin-resistant and -susceptible *Staphylococcus aureus* in the presence of β-lactams on peptidoglycan structure and susceptibility to lytic enzymes. Antimicrob. Agents Chemother. **29**:250–257.
396. **Quie, P. G., M. Collin, and J. B. Cardle.** 1960. Neomycin-resistant staphylococci. Lancet **ii**:124–126.
397. **Ranzini, A. C., and D. T. Dubin.** 1983. The 'erythromycin-resistance' methylated sequence of *Staphylococcus aureus* ribosomal RNA. Plasmid **10**:293–295.
398. **Recsei, P., B. Kreiswirth, M. O'Reilly, P. Schlievert, A. Gruss, and R. P. Novick.** 1986. Regulation of exoprotein gene expression in *Staphylococcus aureus* by *agr*. Mol. Gen. Genet. **202**:58–61.
399. **Reynolds, P. E.** 1984. Resistance of the antibiotic target site. Br. Med. Bull. **40**:3–10.
400. **Reynolds, P. E.** 1985. Inhibitors of bacterial cell wall synthesis. Symp. Soc. Gen. Microbiol. **38**:13–40.
401. **Reynolds, P. E., and D. F. J. Brown.** 1985. Penicillin-binding proteins of β-lactam-resistant strains of *Staphylococcus aureus*: effect of growth conditions. FEBS Lett. **192**:28–32.
402. **Reynolds, P. E., and C. Fuller.** 1986. Methicillin-resistant strains of *Staphylococcus aureus*; presence of identical additional penicillin-binding protein in all strains examined. FEMS Microbiol. Lett. **33**:251–254.
403. **Richmond, M. H.** 1965. Wild-type variants of exopenicillinase from *Staphylococcus aureus*. Biochem. J. **94**:584–593.
404. **Richmond, M. H.** 1965. Dominance of the inducible state in strains of *Staphylococcus aureus* containing two distinct penicillinase plasmids. J. Bacteriol. **90**:370–374.
405. **Richmond, M. H.** 1969. Extrachromosomal elements and the spread of antibiotic resistance in bacteria. Biochem. J. **113**:225–234.
406. **Richmond, M. H.** 1972. A comparison of the exopenicillinase mediated by a chromosomal gene in a strain of *Staphylococcus aureus* PS80 and by a plasmid gene in *Staphylococcus aureus* 8325. Genet. Res. **19**:187–189.
407. **Richmond, M. H.** 1972. Some environmental consequences of the use of antibiotics: or 'what goes up must come down.' J. Appl. Bacteriol. **35**:155–176.
408. **Roberts, A. N., G. S. Hudson, and S. Brenner.** 1985. An erythromycin-resistance gene from an erythromycin-producing strain of *Arthrobacter* sp. Gene **35**:259–270.
409. **Robertson, J. J.** 1963. Neomycin resistance in a newly recognised strain of *Staphylococcus aureus*. Lancet **ii**:333–334.
410. **Robinson, J. B., and O. H. Tuovinen.** 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses. Microbiol. Rev. **48**:95–124.
411. **Rollins, L. D., L. N. Lee, and D. J. LeBlanc.** 1985. Evidence for a disseminated erythromycin resistance determinant mediated by Tn*917*-like sequences among group D streptococci isolated from pigs, chickens, and humans. Antimicrob. Agents Chemother. **27**:439–444.
412. **Rosdahl, V. T.** 1973. Naturally-occurring constitutive β-lactamase of novel serotype in *Staphylococcus aureus*. J. Gen. Microbiol. **77**:229–231.
413. **Rosenblum, E. D., and S. Tyrone.** 1964. Serology, density, and morphology of staphylococcal phages. J. Bacteriol. **88**:1737–1742.
414. **Rosendal, K., J. Bang, and V. T. Rosdahl.** 1981. Gentamicin-resistant *Staphylococcus aureus* strains isolated in Denmark in 1979. Acta Pathol. Microbiol. Scand. Sect. B **89**:185–191.
415. **Rosendorf, L. L., and F. H. Kayser.** 1974. Transduction and plasmid deoxyribonucleic acid analysis in a multiply antibiotic-resistant strain of *Staphylococcus epidermidis*. J. Bacteriol. **120**:679–686.
416. **Rossi, L., E. Tonin, Y. R. Cheng, and R. Fontana.** 1985. Regulation of penicillin-binding protein activity: description of a methicillin-inducible penicillin-binding protein in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **27**:828–831.
417. **Rountree, P. M.** 1959. Changes in the phage-typing patterns of staphylococci following lysogenization. J. Gen. Microbiol. **20**:620–633.
418. **Rountree, P. M.** 1978. History of staphylococcal infection in Australia. Med. J. Aust. **2**:543–546.
419. **Rountree, P. M., and M. A. Beard.** 1965. The spread of neomycin-resistant staphylococci in a hospital. Med. J. Aust. **1**:498–502.
420. **Rountree, P. M., and M. A. Beard.** 1968. Hospital strains of *Staphylococcus aureus*, with particular reference to methicillin-resistant strains. Med. J. Aust. **2**:1163–1168.
421. **Rountree, P. M., and A. M. Vickery.** 1973. Further observations on methicillin-resistant staphylococci. Med. J. Aust. **1**:1030–1034.
422. **Rudin, L., J.-E. Sjostrom, M. Lindberg, and L. Philipson.** 1974. Factors affecting competence for transformation in *Staphylococcus aureus*. J. Bacteriol. **118**:155–164.
423. **Rush, M. G., C. N. Gordon, R. P. Novick, and R. C. Warner.** 1969. Penicillinase plasmid DNA from *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **63**:1304–1310.
424. **Sabath, L. D.** 1977. Chemical and physical factors influencing methicillin resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Antimicrob. Chemother. **3**(Suppl. C):47–51.
425. **Sabath, L. D.** 1979. Staphylococcal tolerance to penicillins and cephalosporins, p. 299–303. *In* D. Schlessinger (ed.), Microbi-

ology—1979. American Society for Microbiology, Washington, D.C.
426. **Sabath, L. D.** 1982. Mechanisms of resistance to β-lactam antibiotics in strains of *Staphylococcus aureus*. Ann. Intern. Med. **97**:339–344.
427. **Sabath, L. D., and S. J. Wallace.** 1971. Factors influencing methicillin resistance in staphylococci. Ann. N.Y. Acad. Sci. **182**:258–266.
428. **Sabath, L. D., S. J. Wallace, and D. A. Gerstein.** 1972. Suppression of intrinsic resistance to methicillin and other penicillins in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **2**:350–355.
429. **Sabath, L. D., N. Wheeler, M. Laverdiere, D. Blazevic, and B. J. Wilkinson.** 1977. A new type of penicillin resistance of *Staphylococcus aureus*. Lancet **i**:443–447.
430. **Sadaie, Y., K. C. Burtis, and R. H. Doi.** 1980. Purification and characterization of a kanamycin nucleotidyltransferase from plasmid pUB110-carrying cells of *Bacillus subtilis*. J. Bacteriol. **141**:1178–1182.
431. **Sands, L. C., and W. V. Shaw.** 1973. Mechanism of chloramphenicol resistance in staphylococci: characterization and hybridization of variants of chloramphenicol acetyltransferase. Antimicrob. Agents Chemother. **3**:299–305.
432. **Saunders, J. R.** 1984. Genetics and evolution of antibiotic resistance. Br. Med. Bull. **40**:54–60.
433. **Schaberg, D. R., D. B. Clewell, and L. Glatzer.** 1982. Conjugative transfer of R-plasmids from *Streptococcus faecalis* to *Staphylococcus aureus*. Antimicrob. Agents Chemother. **22**:204–207.
434. **Schaberg, D. R., G. Power, J. Betzold, and B. A. Forbes.** 1985. Conjugative R plasmids in antimicrobial resistance of *Staphylococcus aureus* causing nosocomial infections. J. Infect. Dis. **152**:43–49.
435. **Schaefler, S.** 1982. Bacteriophage-mediated acquisition of antibiotic resistance by *Staphylococcus aureus* type 88. Antimicrob. Agents Chemother. **21**:460–467.
436. **Schaefler, S., W. Francois, and C. L. Ruby.** 1976. Minocycline resistance in *Staphylococcus aureus*: effect on phage susceptibility. Antimicrob. Agents Chemother. **9**:600–613.
437. **Schaefler, S., D. Jones, W. Perry, T. Baradet, E. Mayr, and C. Rampersad.** 1984. Methicillin-resistant *Staphylococcus aureus* strains in New York City hospitals: interhospital spread of resistant strains of type 88. J. Clin. Microbiol. **20**:536–538.
438. **Schaefler, S., D. Jones, W. Perry, L. Ruvinskaya, T. Baradet, E. Mayr, and M. E. Wilson.** 1981. Emergence of gentamicin- and methicillin-resistant *Staphylococcus aureus* strains in New York City hospitals. J. Clin. Microbiol. **13**:754–759.
439. **Schottel, J., A. Mandal, D. Clark, S. Silver, and R. W. Hedges.** 1974. Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. Nature (London) **251**:335–337.
440. **Schugurensky, A., P. Cahn, G. Osatnik, J. Zorzopulos, A. Ruiz Trevisan, and C. D. Denoya.** 1984. An outbreak of multiply resistant *Staphylococcus aureus* in Buenos Aires. Medicina (Buenos Aires) **44**:8–14.
441. **Schwesinger, M. D., and R. P. Novick.** 1975. Prophage-dependent plasmid integration in *Staphylococcus aureus*. J. Bacteriol. **123**:724–738.
442. **Scott, D. F., D. O. Wood, G. H. Brownell, M. J. Carter, and G. K. Best.** 1978. Aminoglycoside modification by gentamicin-resistant isolates of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **13**:641–644.
443. **Shafer, W. M., and J. J. Iandolo.** 1979. Genetics of staphylococcal enterotoxin B in methicillin-resistant isolates of *Staphylococcus aureus*. Infect. Immun. **25**:902–911.
444. **Shafer, W. M., and J. J. Iandolo.** 1980. Transduction of staphylococcal enterotoxin B synthesis: establishment of the toxin gene in a recombination-deficient mutant. Infect. Immun. **27**:280–282.
445. **Shafferman, A., Z. Shalita, and I. Hertman.** 1978. Cleavage maps of a tetracycline plasmid from *Staphylococcus aureus*. J. Bacteriol. **134**:345–348.
446. **Shalita, Z., E. Murphy, and R. P. Novick.** 1980. Penicillinase plasmids of *Staphylococcus aureus*: structural and evolutionary relationships. Plasmid **3**:291–311.
447. **Shannon, K., and I. Phillips.** 1982. Mechanisms of resistance to aminoglycosides in clinical isolates. J. Antimicrob. Chemother. **9**:91–102.
448. **Shanson, D. C.** 1981. Antibiotic-resistant *Staphylococcus aureus*. J. Hosp. Infect. **2**:11–36.
448a. **Shanson, D. C.** 1986. Staphylococcal infections in hospital. Br. J. Hosp. Med. **35**:312–320.
449. **Shanson, D. C., D. Johnstone, and J. Midgley.** 1985. Control of a hospital outbreak of methicillin-resistant *Staphylococcus aureus* infections: value of an isolation unit. J. Hosp. Infect. **6**:285–292.
450. **Shanson, D. C., J. G. Kensit, and R. Duke.** 1976. Outbreak of hospital infection with a strain of *Staphylococcus aureus* resistant to gentamicin and methicillin. Lancet **ii**:1347–1348.
451. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B resistance transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **164**:782–796.
452. **Shaw, W. V.** 1983. Chloramphenicol acetyltransferase: enzymology and molecular biology. Crit. Rev. Biochem. **14**:1–46.
453. **Shaw, W. V.** 1984. Bacterial resistance to chloramphenicol. Br. Med. Bull. **40**:36–41.
454. **Shaw, W. V., D. G. Brenner, S. F. J. LeGrice, S. E. Skinner, and A. R. Hawkins.** 1985. Chloramphenicol acetyltransferase gene of staphylococcal plasmid pC221. FEBS Lett. **179**:101–106.
455. **Shivakumar, A. G., and D. Dubnau.** 1981. Characterization of a plasmid-specified ribosome methylase associated with macrolide resistance. Nucleic Acids Res. **9**:2549–2562.
456. **Shivakumar, A. G., T. J. Gryczan, Y. I. Kozlov, and D. Dubnau.** 1980. Organization of the pE194 genome. Mol. Gen. Genet. **179**:241–252.
457. **Shivakumar, A. G., J. Hahn, and D. Dubnau.** 1979. Studies on the synthesis of plasmid-coded proteins and their control in *Bacillus subtilis* minicells. Plasmid **2**:279–289.
458. **Silver, S., K. Budd, K. M. Leahy, W. V. Shaw, D. Hammond, R. P. Novick, G. R. Willsky, M. H. Malamy, and H. Rosenberg.** 1981. Inducible plasmid-determined resistance to arsenate, arsenite, and antimony (III) in *Escherichia coli* and *Staphylococcus aureus*. J. Bacteriol. **146**:983–996.
459. **Silver, S., and D. Keach.** 1982. Energy-dependent arsenate efflux: the mechanism of plasmid mediated resistance. Proc. Natl. Acad. Sci. USA **79**:6114–6118.
460. **Sjöström, J.-E., S. Löfdahl, and L. Philipson.** 1975. Transformation reveals a chromosomal locus of the gene(s) for methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **123**:905–915.
461. **Sjöström, J.-E., S. Löfdahl, and L. Philipson.** 1979. Transformation of *Staphylococcus aureus* by heterologous plasmids. Plasmid **2**:529–535.
462. **Sjöström, J.-E., and L. Philipson.** 1974. Role of the ϕ11 phage genome in competence of *Staphylococcus aureus*. J. Bacteriol. **119**:19–32.
463. **Skinner, R., E. Cundliffe, and F. J. Schmidt.** 1983. Site of action of a ribosomal RNA methylase responsible for resistance to erythromycin and other antibiotics. J. Biol. Chem. **258**:12702–12706.
464. **Smith, J. T., and S. G. B. Amyes.** 1984. Bacterial resistance to antifolate chemotherapeutic agents mediated by plasmids. Br. Med. Bull. **40**:42–46.
465. **Smith, K., and R. P. Novick.** 1972. Genetic studies on plasmid-linked cadmium resistance in *Staphylococcus aureus*. J. Bacteriol. **112**:761–772.
466. **Smith, P. F., and B. J. Wilkinson.** 1981. Differential methicillin susceptibilities of peptidoglycan syntheses in methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. **148**:610–617.
467. **Sompolinsky, D., I. Hammerman, O. Assaf, and A. Wodjani.** 1978. Tetracycline resistance antigen from *Staphylococcus aureus*. FEMS Microbiol. Lett. **4**:23–26.
468. **Sompolinsky, D., Y. Zaidenzaig, R. Zeigler-Schlomowitz, and N. Abramova.** 1970. Mechanism of tetracycline resistance in *S.*

aureus. J. Gen. Microbiol. **62**:351–362.
469. **Sorrell, T. C., D. R. Packham, S. Shanker, M. Foldes, and R. Munro.** 1982. Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:344–350.
470. **Soussy, C. J., D. H. Bouanchaud, J. Fouace, A. Dublanchet, and J. Duval.** 1975. A gentamicin-resistance plasmid in *Staphylococcus aureus*. Ann. Microbiol. (Paris) **126B**:91–94.
471. **Speller, D. C. E., D. Raghunath, M. Stephens, A. C. Viant, D. S. Reeves, P. J. Wilkinson, J. M. Broughall, and H. A. Holt.** 1976. Epidemic infection by a gentamicin-resistant *Staphylococcus aureus* in three hospitals. Lancet **i**:464–466.
472. **Spitzy, K. H., and M. Rotter.** 1979. Resistente Staphylokokken neuerlich im Vormarsch? Infection **7**(Suppl. 2):S220–224.
473. **Spratt, B. G.** 1983. Penicillin-binding proteins and the future of β-lactam antibiotics. J. Gen. Microbiol. **129**:1247–1260.
474. **Stahl, M. L., and P. A. Pattee.** 1983. Computer-assisted chromosome mapping by protoplast fusion in *Staphylococcus aureus*. J. Bacteriol. **154**:395–405.
475. **Stahl, M. L., and P. A. Pattee.** 1983. Confirmation of protoplast fusion-derived linkages in *Staphylococcus aureus* by transformation with protoplast DNA. J. Bacteriol. **154**:406–412.
476. **Stewart, G. C., and E. D. Rosenblum.** 1980. Transduction of methicillin resistance in *Staphylococcus aureus*: recipient effectiveness and beta-lactamase production. Antimicrob. Agents Chemother. **18**:424–432.
477. **Stewart, G. C., and E. D. Rosenblum.** 1980. Genetic behavior of the methicillin resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **144**:1200–1202.
478. **Stewart, P. R., H. G. Waldron, J. S. Lee, and P. R. Matthews.** 1985. Molecular relationships among serogroup B bacteriophages of *Staphylococcus aureus*. J. Virol. **55**:111–116.
479. **Stiffler, P. W., H. M. Sweeney, and S. Cohen.** 1973. Absence of circular plasmid deoxyribonucleic acid attributable to a genetic determinant for methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **116**:771–777.
480. **Stiffler, P. W., H. M. Sweeney, and S. Cohen.** 1974. Cotransduction of plasmids mediating resistance to tetracycline and chloramphenicol in *Staphylococcus aureus*. J. Bacteriol. **120**:934–944.
481. **Stiffler, P. W., H. M. Sweeney, M. Schneider, and S. Cohen.** 1974. Isolation and characterization of a kanamycin resistance plasmid from *Staphylococcus aureus*. Antimicrob. Agents Chemother. **6**:516–520.
482. **Stobberingh, E. E., R. Schiphof, and J. S. Sussenbach.** 1977. Occurrence of a class II restriction endonuclease in *Staphylococcus aureus*. J. Bacteriol. **131**:645–649.
483. **Stobberingh, E. E., and K. C. Winkler.** 1977. Restriction-deficient mutants of *Staphylococcus aureus*. J. Gen. Microbiol. **99**:359–367.
484. **Summers, A. O.** 1984. Bacterial metal ion resistances, p. 345–367. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
484a. **Summers, A. O.** 1986. Organization, expression, and evolution of genes for mercury resistance. Annu. Rev. Microbiol. **40**:607–634.
485. **Summers, A. O., and S. Silver.** 1978. Microbial transformation of metals. Annu. Rev. Microbiol. **32**:637–672.
486. **Sutherland, R., and G. N. Rolinson.** 1964. Characteristics of methicillin-resistant staphylococci. J. Bacteriol. **87**:887–899.
487. **Suzuki, I., N. Takahashi, S. Shirato, H. Kawabe, and S. Mitsuhashi.** 1975. Adenylylation of streptomycin in *Staphylococcus aureus*: a new streptomycin adenylytransferase, p. 463–473. *In* S. Mitsuhashi and H. Hashimoto (ed.), Microbial drug resistance. University Park Press, Tokyo.
488. **Swedberg, G., and O. Skold.** 1980. Characterization of different plasmid-borne dihydropteroate synthases mediating bacterial resistance to sulfonamides. J. Bacteriol. **142**:1–7.
489. **Sweeney, H. M., and S. Cohen.** 1968. Wild-type strain of *Staphylococcus aureus* containing two genetic linkage groups for penicillinase production. J. Bacteriol. **96**:920–924.
490. **Sykes, R. B., and D. P. Bonner.** 1984. Counteracting antibiotic resistance: new drugs. Br. Med. Bull. **40**:96–101.
491. **Tennent, J. M., B. R. Lyon, M. T. Gillespie, J. W. May, and R. A. Skurray.** 1985. Cloning and expression of *Staphylococcus aureus* plasmid-mediated quaternary ammonium resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **27**:79–83.
492. **Tennent, J. M., J. W. May, and R. A. Skurray.** 1984. Multiple antibiotic resistance in *Staphylococcus aureus* and *Staphylococcus epidermidis*: plasmids in strains associated with nosocomial infection. Pathology **16**:250–255.
492a. **Tennent, J. M., J. W. May, and R. A. Skurray.** 1986. Characterisation of chloramphenicol resistance plasmids of *Staphylococcus aureus* and *Staphylococcus epidermidis* by restriction enzyme mapping techniques. J. Med. Microbiol. **22**:79–84.
493. **Thabaut, A., M. Meyran, and M. Huerre.** 1985. Evolution and present situation of *Staphylococcus aureus* sensitivity to MLS in hospital (1975–83). J. Antimicrob. Chemother. **16**(Suppl. A):205–207.
494. **Thompson, C. J., and G. S. Gray.** 1983. Nucleotide sequence of a streptomycete aminoglycoside phosphotransferase gene and its relationship to phosphotransferases encoded by resistance plasmids. Proc. Natl. Acad. Sci. USA **80**:5190–5194.
495. **Thompson, C. J., R. H. Skinner, J. Thompson, J. M. Ward, D. A. Hopwood, and E. Cundliffe.** 1982. Biochemical characterization of resistance determinants cloned from antibiotic-producing streptomycetes. J. Bacteriol. **151**:678–685.
496. **Thompson, N. E., and P. A. Pattee.** 1977. Transformation in *Staphylococcus aureus*: role of bacteriophage and incidence of competence among strains. J. Bacteriol. **129**:778–788.
497. **Thompson, N. E., and P. A. Pattee.** 1981. Genetic transformation of *Staphylococcus aureus*: demonstration of a competence-conferring factor of bacteriophage origin in bacteriophage 80α lysates. J. Bacteriol. **148**:294–300.
498. **Thompson, R. L., I. Cabezudo, and R. P. Wenzel.** 1982. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:309–317.
499. **Timoney, J. F., J. Port, J. Giles, and J. Spanier.** 1978. Heavy-metal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbiol. **36**:465–472.
500. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1980. Properties of erythromycin-inducible transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **141**:1366–1374.
500a. **Tonin, E., and A. Tomasz.** 1986. β-Lactam-specific resistant mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **30**:577–583.
501. **Totten, P. A., L. Vidal, and J. Baldwin.** 1981. Penicillin and tetracycline resistance plasmids in *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **20**:359–365.
502. **Townsend, D. E., N. Ashdown, D. I. Annear, and W. B. Grubb.** 1985. A conjugative plasmid encoding production of a diffusible pigment and resistance to aminoglycosides and macrolides in *Staphylococcus aureus*. Aust. J. Exp. Biol. Sci. **63**:573–586.
503. **Townsend, D. E., N. Ashdown, J. M. Bradley, J. W. Pearman, and W. B. Grubb.** 1984. 'Australian' methicillin-resistant *Staphylococcus aureus* in a London hospital? Med. J. Aust. **141**:339–340.
504. **Townsend, D. E., N. Ashdown, L. C. Greed, and W. B. Grubb.** 1984. Analysis of plasmids mediating gentamicin resistance in methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **13**:347–352.
505. **Townsend, D. E., N. Ashdown, L. C. Greed, and W. B. Grubb.** 1984. Transposition of gentamicin resistance to staphylococcal plasmids encoding resistance to cationic agents. J. Antimicrob. Chemother. **14**:115–124.
506. **Townsend, D. E., N. Ashdown, and W. B. Grubb.** 1985. Evolution of Australian isolates of methicillin-resistant *Staphylococcus aureus*: a problem of plasmid incompatibility? J. Med. Microbiol. **20**:49–61.
507. **Townsend, D. E., N. Ashdown, M. Momoh, and W. B. Grubb.** 1985. Distribution of plasmid-borne resistance to nucleic acid binding compounds in methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **15**:417–434.
508. **Townsend, D. E., N. Ashdown, J. W. Pearman, D. I. Annear,**

and W. B. Grubb. 1985. Genetics and epidemiology of methicillin-resistant *Staphylococcus aureus* isolated in a Western Australian hospital. Med. J. Aust. **142**:108–111.

509. **Townsend, D. E., S. Bolton, N. Ashdown, and W. B. Grubb.** 1985. Transfer of plasmid-borne aminoglycoside-resistance determinants in staphylococci. J. Med. Microbiol. **20**:169–185.

509a. **Townsend, D. E., S. Bolton, N. Ashdown, S. Taheri, and W. B. Grubb.** 1986. Comparison of phage-mediated and conjugative transfer of staphylococcal plasmids *in vitro* and *in vivo*. J. Med. Microbiol. **22**:107–114.

510. **Townsend, D. E., L. den Hollander, S. Bolton, and W. B. Grubb.** 1986. Clinical isolates of staphylococci conjugate on contact with dry absorbent surfaces. Med. J. Aust. **144**:166.

511. **Townsend, D. E., W. B. Grubb, and D. I. Annear.** 1985. A plasmid for diffusible pigment production in *Staphylococcus aureus*. Aust. J. Exp. Med. Sci. **63**:463–472.

512. **Townsend, D. E., W. B. Grubb, and N. Ashdown.** 1983. Gentamicin resistance in methicillin-resistant *Staphylococcus aureus*. Pathology **15**:169–174.

513. **Townsend, D. E., W. B. Grubb, and N. Ashdown.** 1983. Genetics of drug resistance in methicillin-resistant *Staphylococcus aureus* from Australian hospitals. J. Hosp. Infect. **4**:331–337.

514. **Trieu-Cuot, P., and P. Courvalin.** 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3′5″-aminoglycoside phosphotransferase type III. Gene **23**:331–341.

515. **Trieu-Cuot, P., G. Gerbaud, T. Lambert, and P. Courvalin.** 1985. *In vivo* transfer of genetic information between Gram-positive and Gram-negative bacteria. EMBO J. **4**:3583–3587.

515a. **Tuomanen, E., D. T. Durack, and A. Tomasz.** 1986. Antibiotic tolerance among clinical isolates of bacteria. Antimicrob. Agents Chemother. **30**:521–527.

516. **Tynecka, Z., Z. Gos, and J. Zajac.** 1981. Reduced cadmium transport determined by a resistance plasmid in *Staphylococcus aureus*. J. Bacteriol. **147**:305–312.

517. **Tynecka, Z., Z. Gos, and J. Zajac.** 1981. Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **147**:313–319.

518. **Ubelaker, M. H., and E. D. Rosenblum.** 1978. Transduction of plasmid determinants in *Staphylococcus aureus* and *Escherichia coli*. J. Bacteriol. **133**:699–707.

519. **Ubukata, K., and M. Konno.** 1982. Gentamicin resistance in *Staphylococcus aureus* isolated in Japan. Part 2. Transduction of drug resistance by phage lysate and analysis of plasmid from gentamicin-resistant *Staphylococcus aureus*. Chemotherapy (Tokyo) **30**:96–103.

520. **Ubukata, K., N. Yamashita, A. Gotoh, and M. Konno.** 1984. Purification and characterization of aminoglycoside-modifying enzymes from *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **25**:754–759.

521. **Ubukata, K., N. Yamashita, and M. Konno.** 1985. Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **27**:851–857.

522. **Uchiyama, H., and B. Weisblum.** 1985. N-Methyl transferase of *Streptomyces erythraeus* that confers resistance to the macrolide-lincosamide-streptogramin B antibiotics: amino acid sequence and its homology to cognate r-factor enzymes from pathogenic bacilli and cocci. Gene **38**:103–110.

523. **Utsui, Y., and T. Yokota.** 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **28**:397–403.

524. **van Boven, C. P. A., E. E. Stobberingh, J. Verhoef, and K. C. Winkler.** 1974. Restriction and modification of phages in staphylococcal phage typing. Ann. N.Y. Acad. Sci. **236**:376–388.

525. **Varaldo, P. E., P. Cipriani, A. Focá, C. Geraci, A. Giordano, M. A. Madeddu, A. Orsi, R. Pompei, M. Prenna, A. Repetto, S. Ripa, P. Rosselli, G. Russo, F. Scazzocchio, and G. Stassi.** 1984. Identification, clinical distribution, and susceptibility to methicillin and 18 additional antibiotics of clinical *Staphylococcus* isolates: nationwide investigation in Italy. J. Clin. Microbiol. **19**:838–843.

526. **Vickery, A. M., M. A. Beard-Pegler, and P. M. Rountree.** 1983. Strain differentiation in methicillin-resistant *Staphylococcus aureus*. Pathology **15**:235–240.

526a. **Vickeny, A. M., M. A. Beard-Pegler, and E. Stubbs.** 1986. Phage-typing patterns and lysogenicity of methicillin-resistant strain of *Staphylococcus aureus* from Sydney, Australia, 1965–85. J. Med. Microbiol. **22**:209–216.

527. **Vogel, L., C. Nathan, H. M. Sweeney, S. A. Kabins, and S. Cohen.** 1978. Infections due to gentamicin-resistant *Staphylococcus aureus* strain in a nursery for neonatal infants. Antimicrob. Agents Chemother. **13**:466–472.

527a. **Waldvogel, F. A.** 1986. Treatment of infections due to methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **7**(Suppl. A):37–46.

528. **Watanakunakorn, C.** 1982. Treatment of infections due to methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:376–378.

529. **Watanakunakorn, C.** 1984. Mode of action and *in-vitro* activity of vancomycin. J. Antimicrob. Chemother. **14**(Suppl. D):7–18.

530. **Waxman, D. J., and J. L. Strominger.** 1983. Penicillin-binding proteins and the mechanism of action of β-lactam antibiotics. Annu. Rev. Biochem. **52**:825–869.

531. **Weinstein, R. A., S. A. Kabins, C. Nathan, H. M. Sweeney, H. W. Jaffe, and S. Cohen.** 1982. Gentamicin-resistant staphylococci as hospital flora: epidemiology and resistance plasmids. J. Infect. Dis. **145**:374–382.

532. **Weisblum, B.** 1984. Inducible erythromycin resistance in bacteria. Br. Med. Bull. **40**:47–53.

533. **Weisblum, B.** 1985. Inducible resistance to macrolides, lincosamides and streptogramin type B antibiotics: the resistance phenotype, its biological diversity, and structural elements that regulate expression-a review. J. Antimicrob. Chemother. **16**(Suppl. A):63–90.

534. **Weisblum, B., M. Y. Graham, T. Gryczan, and D. Dubnau.** 1979. Plasmid copy number control: isolation and characterization of high-copy-number mutants of plasmid pE194. J. Bacteriol. **137**:635–643.

535. **Weisblum, B., S. B. Holder, and S. M. Halling.** 1979. Deoxyribonucleic acid sequence common to staphylococcal and streptococcal plasmids which specify erythromycin resistance. J. Bacteriol. **138**:990–998.

536. **Weiss, A. A., S. D. Murphy, and S. Silver.** 1977. Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. J. Bacteriol. **132**:197–208.

537. **Weiss, A. A., S. Silver, and T. G. Kinscherf.** 1978. Cation transport alteration association with plasmid-determined resistance to cadmium in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **14**:856–865.

538. **Wenzel, R. P.** 1982. The emergence of methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:440–442.

539. **Wilkinson, B. J., and M. J. Nadakavukaren.** 1983. Methicillin-resistant septal peptidoglycan synthesis in a methicillin-resistant *Staphylococcus aureus* strain. Antimicrob. Agents Chemother. **23**:610–613.

540. **Willetts, N., and R. Skurray.** 1980. The conjugation system of F-like plasmids. Annu. Rev. Genet. **14**:41–76.

541. **Williams, R. F.** 1982. Choice of chemotherapy for infection by *Staphylococcus aureus*. J. Antimicrob. Chemother. **9**:1–3.

542. **Wilson, C. R., and J. N. Baldwin.** 1978. Characterization and construction of molecular cloning vehicles within *Staphylococcus aureus*. J. Bacteriol. **136**:402–413.

543. **Wilson, C. R., S. E. Skinner, and W. V. Shaw.** 1981. Analysis of two chloramphenicol resistance plasmids from *Staphylococcus aureus*: insertional inactivation of Cm resistance, mapping of restriction sites, and construction of cloning vehicles. Plasmid **5**:245–258.

544. **Winkler, K. C., and C. Grootsen.** 1961. The relation of phage pattern and lysogenicity in the phage typing of staphylococci of phage-group II. Antonie van Leeuwenhoek J. Microbiol. Serol. **27**:225–246.

545. **Witte, W.** 1977. Transfer of drug-resistance-plasmids in mixed cultures of staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **237**:147–159.

546. **Witte, W.** 1981. Transfer of drug-resistance plasmids in mixed

cultures of staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl. **10**:543–545.

547. **Witte, W.** 1981. Role of phage in the transfer of plasmids in mixed cultures of *Staphylococcus aureus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **249**:195–202.
548. **Witte, W., and K. Dünnhaupt.** 1982. Multiple drug-resistant *Staphylococcus aureus* with resistance to oxytetracycline and minocycline, an example for the distribution of a multiresistant strain-clone. J. Hyg. Epidemiol. Microbiol. Immunol. (Prague) **26**:49–56.
549. **Witte, W., and K. Dünnhaupt.** 1984. Occurrence of a nonplasmid-located determinant of gentamicin resistance in strains of *Staphylococcus aureus*. J. Hyg. **93**:1–8.
550. **Witte, W., L. Green, T. K. Misra, and S. Silver.** 1986. Resistance to mercury and to cadmium in chromosomally resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29**:663–669.
551. **Wood, D. O., M. J. Carter, and G. K. Best.** 1977. Plasmid-mediated resistance to gentamicin in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **12**:513–517.
552. **Wyatt, T. D., W. P. Ferguson, T. S. Wilson, and E. McCormick.** 1977. Gentamicin resistant *Staphylococcus aureus* associated with the use of topical gentamicin. J. Antimicrob. Chemother. **3**:213–217.
553. **Wyke, A. W.** 1984. Isolation of five penicillin-binding proteins from *Staphylococcus aureus*. FEMS Microbiol. Lett. **22**:133–138.
554. **Wyke, A. W., J. B. Ward, and M. V. Hayes.** 1982. Synthesis of peptidoglycan *in vivo* in methicillin-resistant *Staphylococcus aureus*. Eur. J. Biochem. **127**:553–558.
555. **Wyke, A. W., J. B. Ward, M. V. Hayes, and N. A. C. Curtis.** 1981. A role *in vivo* for penicillin-binding protein-4 of *Staphylococcus aureus*. Eur. J. Biochem. **119**:389–393.
556. **Wyman, L., and R. P. Novick.** 1974. Studies on plasmid replication. IV. Complementation of replication-defective mutants by an incompatibility-deficient plasmid. Mol. Gen. Genet. **135**:149–161.
557. **Yamada, T., D. Tipper, and J. Davies.** 1968. Enzymatic inactivation of streptomycin by R-factor resistant *Escherichia coli*. Nature (London) **219**:228–291.
558. **Yourassowsky, E., M. P. van der Linden, M. J. Lismont, and F. Crokaert.** 1981. Combination of minocycline and rifampicin against methicillin- and gentamicin-resistant *Staphylococcus aureus*. J. Clin. Pathol. **34**:559–563.
559. **Zaidenzaig, Y., J. E. Fitton, L. C. Packman, and W. V. Shaw.** 1979. Characterization and comparison of chloramphenicol acetyltransferase variants. Eur. J. Biochem. **100**:609–618.